OBITUARIES

JOHN DYBHAVN

In 1923 the Department of Fisheries appointed two members to the then Biological Board of Canada as direct representatives of the fishing industry. One of these was Mr. John Dybhavn, of Prince Rupert, B.C. When the Board was reconstituted as the Fisheries Research Board in 1937, Mr. Dybhavn was appointed to the new Board for a five-year term, and he was reappointed for a further term early in 1943. Throughout this long period he acted as Chairman of the Pacific Sub-Executive Committee of the Board, and, in spite of illness, he was on his way south to attend a meeting of this committee when he became so seriously ill that he had to return, and he died shortly after reaching Prince Rupert on April 3rd, 1943. He is survived by his widow, a son, Lieutenant George Dybhavn, R.C.A., and a daughter.

John Dybhavn was born in Bergund, Norway, on April 18th, 1879. After attending public school he entered the Military School for Infantry at Bergen, graduating from it in 1899. After some years in business in Aalesund, he emigrated in 1907. He went first to Seattle, and then, in 1908, to Prince Rupert as a contractor in railway construction. In 1911 he started a real estate and insurance business, with which Mr. Olof Hanson, now M.P. for Skeena, very shortly afterwards became associated. In 1916 the Royal Fish Company was

organized, and he was appointed its manager, retaining this position until his death. For a while also he headed the Massett Canners Company in the Queen Charlotte Islands.

He became a naturalized Canadian citizen in 1911, and rapidly became one of Prince Rupert's leading citizens. He was elected an Alderman in 1912, serving as such for ten years. He was a past president of the Prince Rupert Chamber of Commerce and Rotary Club, a member of the local branch of the Canadian Fisheries Association, and an active member of the Masonic order. He was for many years a leading member of the Prince Rupert Pioneers Association.

In 1918 he was appointed Royal Vice-Consul for Norway for Northern British Columbia, and his valuable services for very many years to the countrymen of his birth were recognized in 1939, when he was awarded the Order of St. Olaf by King Haakon of Norway.

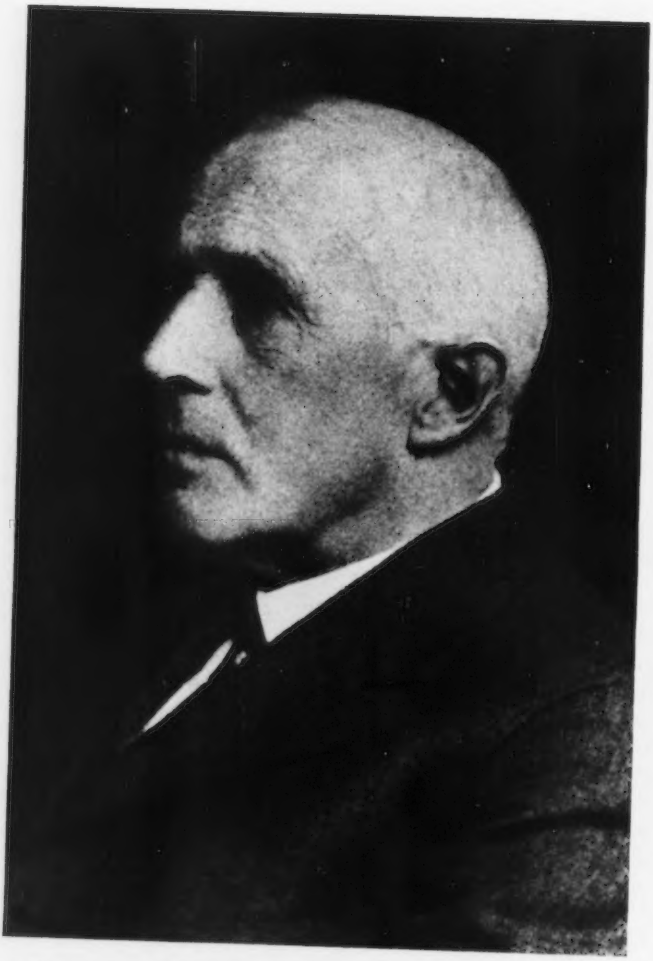
The appreciation of his own community is voiced by an editorial in the Prince Rupert *Daily News* which reads in part: "Were Prince Rupert at any time in the last quarter of a century to have been asked to choose a best citizen, John Dybhavn would have undoubtedly been an outstanding candidate for such an honour. His able and generous services for the public benefit were of a very varied character and were carried out from many angles. . . . Of sound and clear judgment, and keen and practical in a business way, he was one of the pioneers who found it possible to adjust himself to changing conditions as those through which we now pass. . . . He was the kind of citizen we can ill afford to lose."

The Biological Board in its early days tended to be somewhat academic in its approach to the problems of the Industry. The experiment of bringing representatives of the Industry into close association with the scientific members of the Board has been an unqualified success, and the gradual and smooth transfer of its activities so that they are now entirely coordinated to meet the needs of the Department and of the Industry are very largely due to the influence of the late Mr. John Cowie and of Mr. Dybhavn.

As Chairman of the Pacific Sub-Executive Committee Mr. Dybhavn established and maintained a close personal—indeed a paternal—relationship with the staffs of the two Pacific stations of the Board, and they learned from experience that they could depend completely on his assistance and advice at all times.

The writer was associated with Mr. Dybhavn in the work of the Board during the past sixteen years. He helped us always to keep to the main problem, the needs of the Industry and how we could help. His counsel was always wise, his judgment sound, and we could rely on both. He learned easily to appreciate the scientific point of view, and taught us, the scientists, to appreciate the point of view of the Industry, and while so doing became the personal friend of all of us. We shall greatly miss his advice and counsel.

A. T. Cameron,
Chairman, Fisheries Research Board,
August, 1943.



Professor Arthur Willey, D.Sc., F.R.S.C., F.R.S.

Dr. Arthur Willey (1867-1942) was Professor of Zoology at McGill University, an ardent investigator of the origin of vertebrate animals, an intrepid explorer of animal life in the East Indies, in Ceylon and along the Pacific and Atlantic coasts of Canada, and was an esteemed pioneer biologist of Canada. Born at Scarborough, England, son of Reverend William Willey, he received his early education at Kingswood School, Bath, and later in Lancaster's research laboratory, University of London.[1] While tutor at Columbia University, 1892-94, he completed his treatise on *Amphioxus* which was published with a preface by Henry Fairfield Osborn. "*Amphioxus and the Ancestry of Vertebrates* caused almost a revolution."[2] He received the D.Sc. degree, London, in 1894.

As Balfour student of Cambridge University, 1894-99, he explored islands in the Southwest Pacific and with the help of natives, partly civilized by "the warlike missionary George Brown"[2], he trapped the pearly Nautilus, first at Blanche bay, now Rabaul, New Britain, as they fed at fifty fathoms upon the submerged slopes of a recent volcanic island. Along the shores, inhabited by the flesh-eating natives of New Guinea, his collections were made under the most hazardous conditions. The material collected formed the basis for Willey's papers (a) on the naturally armoured, submarine-like mollusc, *Nautilus;* (b) on the most archaic of living representatives among worm-like forms which "migrated from the water and colonized terra firma, the somewhat caterpillar-like *Peripatus*," which has "pointed the way onward to their distant and more evolved relatives, the insects"[3]; (c) on the worm-like *Enteropneusta* which "have gill slits opening from the food canal, survivors of primitive forms that diverged near the origin of Chordate stock"; (d) on *Ctenoplana* which "crawls like a flatworm and swims like a ctenophore." He specialized in transition forms. Willey's later and unfruitful search for Nautilus in New Guinea led him to New Caledonia and the New Hebrides. Specialists in various fields of Zoology described the specimens he collected in "Zoological Results", Cambridge, 1898-1902, which comprised six volumes and more than thirty memoirs. The list of authors includes: Ashworth, Beddard, Bedford, Bell, Borradaile, Boulenger, Collinge, Gadow, Gardiner, Hickson, Hiles, Lister, Mayer, Pocock, Pratt, Punnett, Sharp, Shipley, Stebbing, Thornley. Willey's "anatomical account of Nautilus is classical".[2] He received the Hon. M.A. degree of Cambridge in 1898, and was elected to the Royal Society of London.

On his return to England from the East Indies, 1898, Willey became lecturer in Biology at Guy's Hospital, London. "He did not like this soul-killing job and social conditions in England were to him unpleasant while the climate was

[1]Data contributed by Professor D. L. Thomson.

[2]Quotations from "Obituary—Arthur Willey, F.R.S.", *Nature*, January 30th, 1943, by Professor J. S. Gardiner.

[3]Quotations from J. Arthur Thomson, "Biology for Everyman."

anathema."[2] For these he exchanged, first the tropical Colombo, Ceylon, and later the snowy winters of Montreal, relieved by summers at coastal stations.

As Director of the Museum at Colombo, Ceylon (1902-1910) Willey established a scientific collection and founded the quarterly journal *Spolia Zeylanica*. Reports were published on sea and fresh water fisheries. The report on the windowpane oyster *Placuna placenta* is most notable.

In Canada, Willey took interest in the work of the Biological Board, later to become the Fisheries Research Board, and spent many summers at its Stations, chiefly at St. Andrews, New Brunswick. In 1914, with headquarters at the Pacific Station, Nanaimo, Willey initiated the investigation of the life history of the halibut and of the halibut fishery in the Northern Pacific. In the published account of his work (Contr. Canad. Biol. 1914-1915, pp. 1-17, 1916), several recommendations were made which since that time have been acted upon to the distinct benefit of the halibut fishery. Willey's later papers on marine biology dealt chiefly with plankton forms, notably copepods and polychaete worms.

Although Willey was reserved toward his students, his lectures were made interesting by his rich experience of investigation and exploration and were motivated and unified by the transition-form concept of systematic zoology. His papers on "Convergent evolution" and "Lectures on Darwinism" summarize his viewpoint.

Willey was Professor of Zoology, McGill University from 1910 to 1932 and Emeritus Professor until his death in 1942; he was also a member of the Executive of the Biological Board of Canada, and a fellow and President of Section V (1922-23) of the Royal Society of Canada.

It seems probable that Willey was cognizant of a certain sympathetic attraction toward the Nautilus which he admired, and possibly emulated, subconsciously: the passage from one chamber of abode to another, each acquiring something of the former, although distinct, and each successively somewhat larger and inhabited for a longer period; the ability to withdraw into an artistic shell of silence and mental nourishment, at great depths; and at will, seemingly with or without external stimulation, to strike forth with a wealth of expansive buoyancy and reserve energy, an activity the more impressive since the least expected and resulting in environmental movements and sometimes disturbance. It is probable that a life lived under four sovereigns was recognized by him as representing, in the later years, "the survivors of an almost extinct race of long and honourable pedigree"—the true Victorian.

Arthur Willey is survived by Constance Boyd Willey, who was a constant complement to her husband, and by a brother, W. H. Willey, Cambridge, England.[1]

A. H. Hutchinson.

Vancouver, September 7, 1943.

Dr. C. McLean Fraser and Dr. W. A. Clemens have supplied data and have read the manuscript.

Structure, Deposition and Quality of Oyster Shell (*Ostrea virginica* Gmelin)

By J. C. MEDCOF
(*Prince Edward Island Biological Station*)

(*Received for publication April 22, 1943*)

ABSTRACT

Low quality shells are easily broken, have a high water content and low specific gravity, and contain little pearly and much chalky material. Pearly shell is secreted throughout the season before and after the chalk which it incloses. Chalky areas occur in all shells, have functional importance, are commonest in oysters that are poorest in late summer and are regular in their order of appearance and position on the shell. They are composed of large vertical calcite crystals in a porous mass of smaller crystals with a protein matrix.

It is common knowledge that damage to shells of oysters during handling and shipping affects their keeping quality and that some stocks of oysters are more liable to breakage than others. Such oysters are usually irregular and distorted in shape. Experience in crushing shells for poultry feed has shown that the easily broken shells give a poor yield of the marketable product and a high proportion of an unsaleable fine white dust. If these shells are not thoroughly dry when ground they produce a white paste that clogs the crushing machinery. Such difficulties are not met in grinding hard shells. Finally, practice has shown that the transfer of oysters with poor shells to new areas for a few months may greatly improve the sanitary condition and fatness of their meats, but has little or no effect on the strength and shipping qualities of their shells.

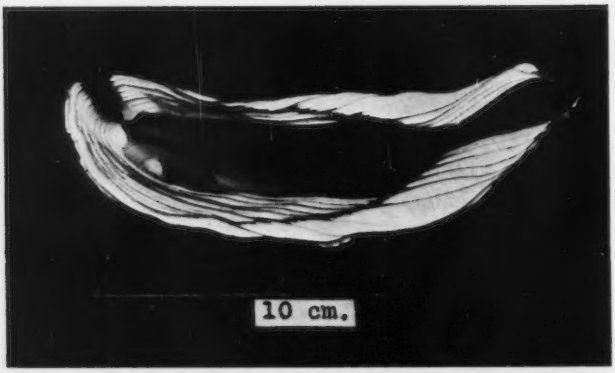
The quality of oyster shells is thus of practical importance and is apparently dependent on structure. Other than this general observation little has been recorded in the literature.

STRUCTURE

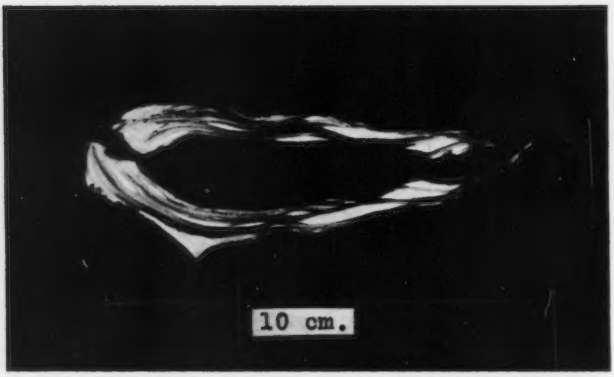
For study, specimens of oyster shells from several inlets and from different parts of these inlets were prepared according to standard petrological techniques. Cut polished surfaces revealed gross features and thin transparent sections examined microscopically showed the finer structure. Decalcification of sections and shell fragments and chemical tests of shell parts were also carried out.

These preparations show that the shell normally consists of two types of secretion systematically related (fig. 1). These are hard pearly shell and soft chalky shell.

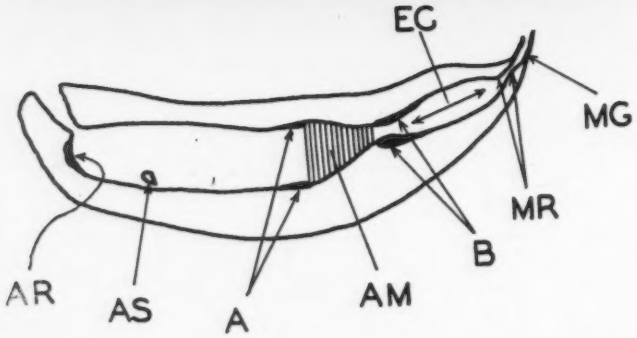
J. FISH. RES. BD. CAN. **6** (3) 1944.
Printed in Canada



A



B



C

FIGURE 1. Polished faces of typical oyster shells sectioned frontally through the region of the adductor muscle scars to show gross structure. A: Shell from Gillis cove, Bras d'Or lakes, N.S. B: Shell from the Wilbur bed, Shediac bay, N.B. C: Diagram to illustrate certain features of the photographs:—*A*, positions of "A" deposits; *AM*, position occupied by adductor muscle; *AR*, anterior recess; *AS*, scar of pedal retractor muscle; *B*, positions of "B" deposits; *EC*, "excurrent chamber" of Orton and Amirthalingam; *MG*, marginal groove; *MR*, marginal ridges.

PEARLY SHELL

Pearly shell is disposed in continuous strata of varying thickness (gray or black lines in fig. 1, A and B). It is hard and variously coloured—transparent-white, yellow, brown or purple. Its surface may be glossy or dull. Schmidt (1931), Douvillé (1936) and others have shown that the strata are laminated and composed of compactly and regularly arranged crystals of calcite set in a protein matrix.

The delicate organic matrix ("animal basis" of Carpenter 1845), which is left when pearly shell is decalcified in weak acid, is reported to be protein, and, on testing, it was found to react positively to Millon's and the biuret tests for protein.

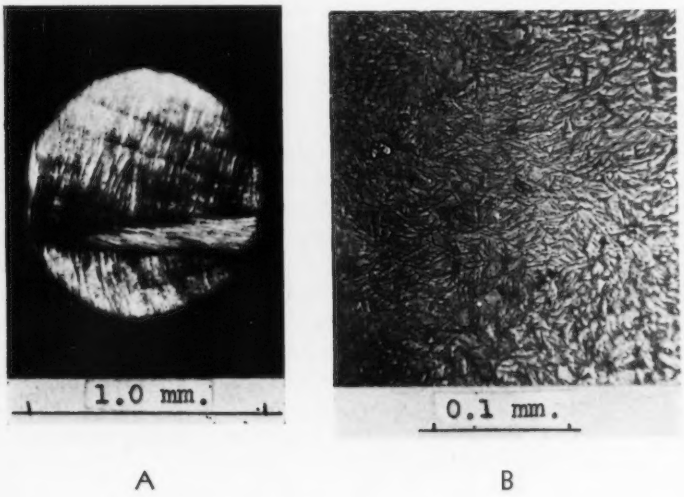


FIGURE 2. Photomicrographs of thin sections of shell, using a polarizing lens with its axis "crossed" with those of the calcite crystals in the sections. A: Section perpendicular to shell face through two laminated chalky deposits separated by pearly shell. B: Section cut parallel to the shell face through a chalky deposit.

CHALKY SHELL

Chalky shell (white in fig. 1, A and B) may be disposed in extensive strata or in isolated pockets of varying size and shape. There may or may not be evidence of lamination within any one deposit. The chalk is so soft that it can be scratched with the fingernail. It is always opaque-white in colour and of a dull velvety lustre.

Tests performed during this study showed that chalky shell has a protein base. Fragments digested in weak hydrochloric acid left no definite membranes of the sort resulting from the digestion of pearly shell but only transparent colourless mucilaginous blobs. These reacted positively to Millon's and the biuret tests for proteins. This finding does not agree with the generally accepted statement of Carpenter (1845) that there is no organic base binding the chalk.

The chalky layers are merely known to contain vertically placed elements (Bøggild 1930, Douvillé 1936) and to be porous (Bøggild 1930). On examination with polarized light, the vertical elements were seen in sections (fig. 2 A) to be long dark pillar-shaped crystals or aggregates of similarly oriented crystals of calcite. They ordinarily extend through the full thickness of the deposits in which they occur, and are set in a matrix of smaller calcite crystals whose axes are not uniformly oriented (fig. 2 B). Among the crystals there are spaces which give the shell its porous character.

SECRETION

The course of shell secretion has been studied in oysters taken from the Bras d'Or lakes, N.S., Malpeque bay, P.E.I., and Shediac bay, N.B., whose condition in relation to temperature and salinity has been reported upon (Medcof and Needler 1941).

Pearly Shell

In all cases the shell interior had a hard pearly surface layer in early spring. Medially this was dull white except for an occasional yellow blotch; outside the marginal ridge (fig. 3) it was glossy white.

After the water temperature rose to 10°C. the secretion of pearly shell began; a narrow band of fragile new shell appeared on the left valve; the inner surface of the shell became highly glossy all over and a reddish-brown blotch appeared posterior to the adductor muscle. While the temperature ranged between 15 and 20°C. this margin grew rapidly, but the appearance of the inner surface remained unchanged, except for chalky deposits, until after spawning.

When the high summer water temperatures began to decline, irregular, highly glossy, yellow blotches became common and expanded until they covered about half the inner surface of the shell.

In the fall when the temperature fell to 15°C. the glossiness disappeared except from the marginal region. This was due to the formation of a thin, easily-scratched, velvety-white, chalky film over the whole central face of the pearly shell. It overlay and obscured much of the yellow area.

When the water temperature fell to 10°C. the inner face was covered by a hard dull film that could scarcely be scratched even with a knife blade and the yellow area was further reduced. The marginal region was still glossy. In this condition the shell remained until the following spring.

In Gillis cove (Bras d'Or lakes, N.S.) these processes were all delayed, probably because of low salinities. The glossiness did not appear until after spawning and the period of marginal shell growth was short. The pearly strata were always very thin (fig. 1) and fragile even at the shell margin.

In studying transparent sections of oyster shell yellow regions were found in the lamellae of the pearly strata. These indicate the positions of the yellow blotches just described. It is suggested that the other changes in the shell face that have been outlined are related to the variations in crystalline structure of pearly shell described in part by other investigators.

Chalky Shell

SEASON

Secretion of chalky shell was limited to a short spring and summer season. The first chalk appeared in small isolated patches deposited upon the glossy surface. These grew in size gradually until after spawning, when there was a rapid increase in their diameter and thickness, sometimes resulting in their confluence. In Gillis cove chalk development was so rapid and extreme that practically the whole internal surface of the shell was covered, sometimes to a depth of two or three millimetres.

When pearly shell was developing its yellowness the chalky deposits began to be covered by pearly shell. Covering was usually complete by the time the autumnal water temperatures fell to 15°C.

The observations showed that shells with extensive chalky deposits typically have thin pearly layers (fig. 1).

DISTRIBUTION

For the stocks studied records were kept of the positions of all chalky secretions from the time they first appeared in spring until they were covered by pearly shell in autumn. Observations made in 1941 on oysters from the Poirier bed, Shediac bay, N.B., are used here as a basis for discussion. The other records accord with these data.

Eleven samples from the Poirier bed were examined between June 7, the date when chalk first appeared, and September 13, when covering was complete. These contained altogether 187 adult oysters, of which 159 showed a total of 716 exposed chalky deposits. The distribution of these in various arbitrarily chosen sectors of the shell is shown in figure 3. The numbers in the figure indicate the percentages of the 159 oysters that showed chalky deposits in the positions indicated. The numbers outside the shell margins refer to deposits found lateral to the marginal ridge (shown in black). Those inside refer to deposits medial to the ridge. The medial deposits A and B (hatched in fig. 3) were so conspicuous and characteristic that their frequencies were listed separately from all others. The positions of the principal axes according to Nelson (1938) are indicated by the straight broken lines.

SIGNIFICANCE

"A" was the first chalky deposit to form in the spring. It appeared in the long narrow groove left at the anterior end of the adductor muscle scar after the muscle had moved posteriorly during spring growth. It was often covered by pearly shell earlier than other deposits so the percentages shown for it in figure 3 do not indicate its true relative frequency. Deposits resembling "A's" were observed in other sharp depressions such as those where the pedal retractor muscle attaches (Leenhardt 1923); those about blisters caused by the annelid *Polydora*, and those found at the margins of partitions formed during "chambering" of shells. Smoothness of shell interior is maintained by such secretions.

"B" was the next deposit to appear and was the most frequent of all. It provides an even surface over which the adductor muscle retreats during growth

and also maintains the constriction shown in figure 1, between what Orton and Amirthalingam (1927) call the "excurrent chamber" and the rest of the shell cavity. This chamber is produced by a wide shallow groove (dotted lines in fig. 3) in each valve which in life accommodates the bulge in the body produced by the true excurrent chamber. Another and lesser groove is found just dorsal to the muscle scar.

Deposits were common in regions of the shell where water leaves and enters, and were shaped and placed in such a way as to indicate that they facilitated the flow of water. In the postero-dorsal or excurrent region they were particularly common and deposited so as to maintain a flatness of the shell surface there. They were also common in the ventral or incurrent sector.

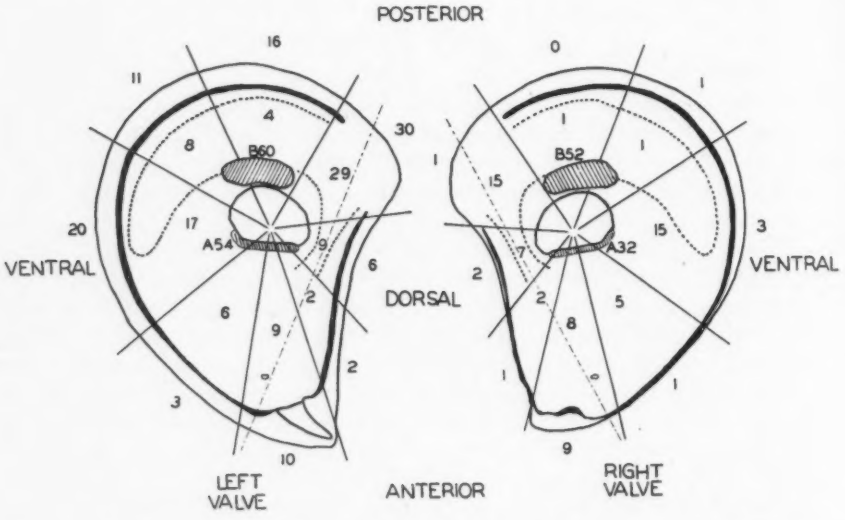


FIGURE 3. Distribution of chalky deposits in various sectors of the inner face of an oyster shell.

A deposit that formed more frequently than the data for the Poirier bed alone would indicate, was in the anterior recess (fig. 1 C) just anterior and lateral to (below) the hinge articulation in the left valve. Practically all the marginal deposits in the anterior sector of the left valve are in this position. The oyster fills in this pocket during growth in much the same fashion as a growing snail fills in the smaller whorls of its shell (Medcof 1940). As the oyster grows the curvature of this part, and indeed of the whole inner surface of the shell, has to be reduced to suit the enlarged body and chalky depositions serve this end.

The deposits in the Gillis cove oysters were so extensive that they produced the same effects as the chambering reported by Orton and Worsnop (1923) and Leenhardt (1926), that is the reduction in size of the shell cavity to accommodate a thin and shrunken body.

STRUCTURAL VARIATION

Biological Basis

It is clear that chalky deposits are normal parts of all oyster shells and that they have functional importance. They help to maintain certain topographical features of the shell interior, preserve a size relationship between meats and shell cavity and regulate the curvature of the inner face of the shell throughout the oyster's life.

Leenhardt (1926) thought that the mantle had to be in close contact with the shell face in order to lay down the delicate organic matrix prerequisite to the formation of pearly shell and that chalky shell was formed as a make-shift, in depressions of the shell face where this contact was impossible. The present observations show that chalky areas like the "B" deposits (fig. 3) may form on prominences of the shell face and that whole sheets of chalk may form, as in Gillis cove shells, regardless of contours. At best, therefore, Leenhardt's explanation may be considered as only partial.

Orton and Amirthalingam (1927) believed that chalky shell could be secreted more quickly than pearly and that it was formed when there was a need for rapid shell development. Havinga's work (1928) on rate of secretion of pearly shell and the present findings, when related to condition studies, suggest a different explanation at least for the large Gillis cove deposits. It was shown above that chalky shell is less compact in structure than pearly; that its greatest development occurred in mid-summer when the oysters were thinnest (Medcof and Needler 1941) and that its development was most extreme in Gillis cove oysters that became the poorest of all stocks examined. No explanation is proposed here for all the types of chalky deposits that have been observed, but it is suggested that the need which poor oysters must experience for economy of shell-building materials is the probable cause for extensive secretions (like those at Gillis cove) of chalky rather than pearly shell.

Physical Properties

It has been shown that, unlike pearly shell, chalk is porous. For this reason the specific gravity of chalky shells is low. For the chalky Gillis cove shells it averaged 1.9; for the hard Wilbur bed (Shediac bay, N.B.) shells, 2.3. Figures 1 and 3 show that chalk is commoner in the lower (left) valve than in the upper. Many determinations for individual valves of Wilbur bed shells show that the average specific gravity of lowers is 2.2; of uppers, 2.4.

The porosity of the chalk also has the effect of raising the water-holding capacity of shells. Thus, after a year's air-drying in the laboratory the specific gravity of Gillis cove shells fell 13%, of Wilbur bed shells 1%.

To test the capacity for water absorption, air-dried shells were cut into pieces about 3 cm. square. Some of the pieces of Gillis cove shells were so light that they floated in water and gained greatly in weight due to water absorption. In contrast, pieces of Wilbur bed shells always sank and absorbed little water.

The chalky shells proved to be the weak shells. Even thick ones from the Scoudouc river, N.B., and Gillis cove could often be broken in the hands. The margins were easily broken and composed of thin fragile pearly laminae, a feature which has been shown to be complementary to extreme chalkiness.

In crushing tests it was found that the chalk broke into a fine powder when too dry or worked down into a paste when too wet. It was the pearly shell that fractured to produce the bulk of the chips that would be suitable for poultry food.

DISCUSSION

It is clear that weak, light and usually irregular shells of low quality differ structurally from those of high quality in having more chalk and less pearly material. Oysters with meats of poor quality had shells of poor quality. This held true in comparisons of oysters from different districts and also for oysters from different parts of the same district. Low salinity seems to be important in the production of very chalky shells but its control is probably indirect through its influence on condition. Water temperature probably exercises a similar indirect control but its influence is probably more intricate since it seems to affect both the rate and the type of shell secretion.

Practice has shown that relaying of poor quality oysters for a few months in waters favourable to fattening brings about a great improvement in condition of meats. Shell quality is only slightly or not at all affected by such treatment. It depends on structure which is unalterable when once established. It would require several years' secretions of thick pearly strata to strengthen a weak chalky shell. Thus the only ready sources of strong-shelled oysters are those areas where shells are naturally of high quality.

REFERENCES

BØGGILD, O. B. The shell structure of the mollusks. *K. danske Vidensk. Selsk. Skr. Naturv.* **9**, Raekke 2 (2), 235-326, 1930.

CARPENTER, W. On the microscopic structure of shells. Part 1. *Rep. Brit. Ass. Adv. Sci.* **1844**, 1-24, 1845.

DOUVILLÉ, H. C. Le test des Lamellibranches, sa formation dans l'*Ostrea edulis*. *C. R. Acad. Sci. Paris*, **203** (20), 965-968, 1936.

HAVINGA, B. The daily rate of growth of oysters during summer. *J. Conseil*, **3**, 231-245, 1928.

LEENHARDT, H. Sur la présence d'un muscle pédieux chez les Ostréidés. *Bull. Soc. Zool.*, **48**, 379-380, 1923.

Quelques études sur *Gryphea angulata* (Huître du Portugal). *Ann. Inst. Océan.* **3**, 1-89, 1926.

MEDCOF, J. C. On the life cycle and other aspects of the snail *Campeloma* in the Speed river. *Canad. J. Res.*, **D18**, 165-172, 1940.

MEDCOF, J. C., AND A. W. H. NEEDLER. The effect of temperature and salinity on the condition of oysters (*Ostrea virginica*). *J. Fish. Res. Bd. Can.*, **5**(3), 253-257, 1941.

NELSON, T. C. The feeding mechanism of the oyster. I. On the pallium and the branchial chambers of *Ostrea virginica*. *O. edulis* and *O. angulata*, with comparisons with other species of the genus. *J. Morph.*, **63**(1), 1-61, 1938.

ORTON, J. H., AND C. AMIRTHALINGAM. Notes on shell depositions in oysters with a note on chemical composition of "chalky deposits" in shells of *O. edulis*. *J. Mar. Biol. Ass.* **14**(4), 935-954, 1927.

ORTON, J. H., AND E. WORSNOP. The cause of chambering in oysters and lamellibranchs. *Nature*, London, **111**, 15, 1923.

SCHMIDT, W. T. Uber die prismenschicht der schale von Ostrea edulis L. *Z. Morph. Ökol. Tiere*, **21**, 789-805, 1931.

Physical Characteristics and Behaviour of Pink Salmon Fry at McClinton Creek, B.C.

By A. L. Pritchard
Pacific Biological Station
(*Received for publication June 18, 1943*)

ABSTRACT

High temperatures appear to shorten and low temperatures lengthen the incubation period of pink salmon (*Oncorhynchus gorbuscha*) eggs. The fry migrate to sea during spring when the yolk sac is almost completely absorbed. There is no significant difference from year to year in qualitative description or in certain countable physical characters. Migration is usually swift and vigorous. The effect of light is demonstrated by the fact that movement is limited to the hours of darkness and slowed by direct moonlight. Rainfall does not initiate migration but causes fry already in motion to proceed more quickly. Small temperature variations have little effect. Oxygen content and pH of the water vary coincidentally with rainfall.

During the investigation of the natural propagation of the pink salmon, *Oncorhynchus gorbuscha*, at McClinton creek, Queen Charlotte islands, British Columbia, from 1930 to 1942, considerable information has been accumulated concerning the fry. This has now been summarized and is set forth in the present paper.

METHODS

Many of the facts relative to the length of the incubation period were procured by direct observation of the spawning redds, many of which were opened so that the eggs and fry might be examined. At intervals throughout the run inspections of the streams were made to determine, if possible, the location and condition of the young salmon. Readings of water temperature close to the bottom and of water height were registered by a thermograph and depth recorder during certain incubation, hatching and migration periods. Oxygen determinations were made by the Winkler method and the pH of the water was taken colorimetrically through the use of La Motte indicators.

Data on the time of migration and on the effect of various climatic factors upon this time were mainly obtained through the operation of a counting fence. This structure was similar in many respects to that installed by Foerster (1929, pp. 4-7) for sockeye salmon at Cultus lake, British Columbia, but exhibited certain modifications designed to suit the different environment.

J. Fish. Res. Bd. Can. 6 (3) 1944.
Printed in Canada.

In order to prevent under-scouring, the strong wooden frame was set on a solid plank platform, 12 ft. (3.7 m.) in width, which in turn rested upon and was securely nailed to three heavy log sills (fig. 1). These sills extended well under both banks of the stream where they were anchored in place by heavy cribbings of logs filled with rocks and gravel. The platform was sealed on both upstream and downstream sides with tongued and grooved sheet piling. There thus resulted a base which was practically watertight and certainly immovable.

The fence superstructure, set back approximately 18 in. (45.7 cm.) from the front edge of the platform, was constructed of heavy rough fir timbers mainly 6 in. by 8 in. (15 cm. by 20 cm.) and 4 in. by 4 in. (10 cm. by 10 cm.). The face of this superstructure sloped at approximately thirty

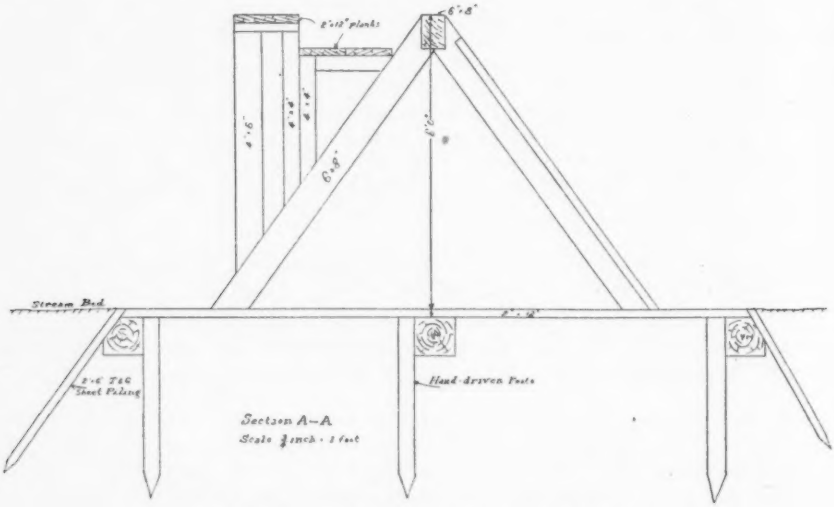


FIGURE 1. Elevation of fry-counting fence at McClinton creek.

degrees from the vertical and was divided into six large sections, 8 ft. (2.4 m.) in width and one small one, 2 ft. (0.6 m.) wide. On the sides of the "A" frames bordering the sections were suitable plates to support the screen sashes. These sashes, 8 ft. by 6 ft. (2.4 m. by 1.8 m.), slid down the plates and rested on the platform in front of a 0.5 in. (1.3 cm.) board. When they were in place, the upstream faces were flush with those of the fence timbers. Although the bottoms of the sashes were bevelled, 2 in. (5 cm.) strips of thick window felting were nailed on each, flush with the upstream edge, to compensate for any minor irregularities in the platform. The screens covering each sash opening (fig. 2) were of galvanized wire 0.072 in. (0.18 cm.) in diameter, 6 to the lineal inch (2.5 cm.). The openings were thus 0.095 in. (0.24 cm.) in width or sufficiently small to retain all salmon fry. To cover the slits between the screen sashes and the timbers

and thus avoid the entry of young salmon, 0.5 by 4 in. (1.3 by 10 cm.), boards were held in place by wooden "buttons" (fig. 2).

The fence installed at an angle of approximately twenty degrees to the stream current (fig. 3), directed the flow toward the downstream corner. Here an oblong trap approximately 16 ft. (4.9 m.) by 8 ft. (2.4 m.) was built parallel to the cribbing on the river bank. The lower end of this pen was closed by two large and one small screens arranged in the form of a V. Division of the trap into two compartments was accomplished in the same manner. When the fry were migrating, the upstream opening of the pen was occupied by a screen of wooden pickets which served to prevent the entry of the larger predators, trout, char, etc., into the enclosed area where the young salmon were concentrated.

The remaining construction in and around the fence was directed mainly toward supporting the structure in the heavy and sudden freshets so com-

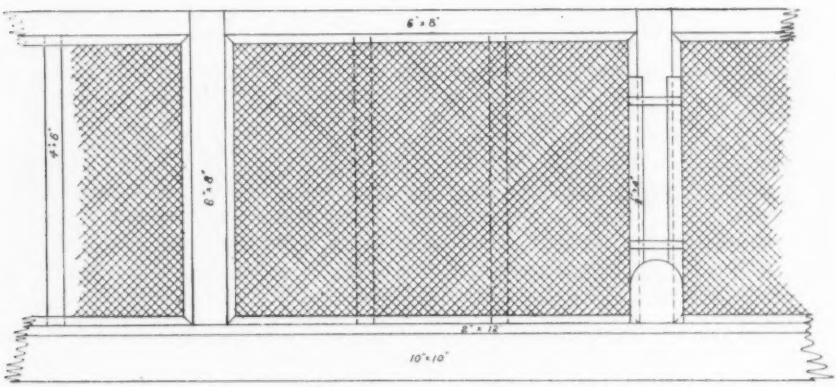


FIGURE 2. Plan of screen installation in fry-counting fence at McClinton creek.

mon in short coastal streams and in allowing easy access to the various locations. Behind each screen rough fir timbers 4 in. by 4 in. (10 cm. by 10 cm.), were set up at the same angle to the platform as the "A" frames, to afford additional protection against "bellying" and breaking. Behind the top of the fence and on the pen a plank walk was constructed.

When the apparatus was in place, fry migrants proceeding downstream came into contact with the galvanized screening. They then worked their way along the structure and entered the pen between the wooden pickets. From the trap they might be dipped, sampled, counted or examined as desired.

In order to check the speed of migration of the young fish and to determine the effect of various climatic conditions on this movement, special marking experiments were conducted. Numbers of fry were taken at the fence and rendered distinguishable by passing silk threads of chosen colours through the back just under the dorsal fin. After retention in

hatchery troughs for at least twenty-four hours to make sure that the "threading" manipulation had not injured them or impaired their reactions, they were carried upstream varying distances and released at night. Recoveries were procured on succeeding days at the fence.

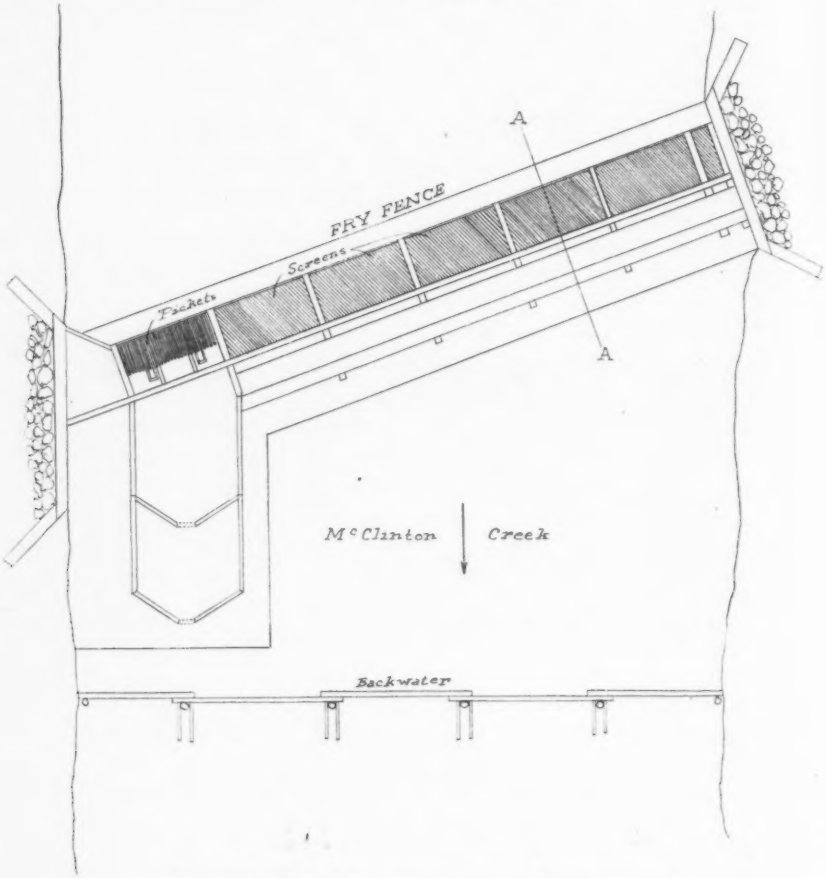


FIGURE 3. Plan of fry-counting fence at McClinton creek.

RESULTS

LENGTH OF INCUBATION PERIOD

The length of the period of incubation of pink salmon eggs has been determined by redd examination and by rearing in a hatchery supplied by creek water. Hatching takes place in the gravel from two to three and one-half months after fertilization or deposition. The yolk-sac fry or alevins which result may remain in the redds as long as three and one-half months, during which time they subsist on the yolk material which is thus gradually

absorbed. When this yolk has almost disappeared, the fry work their way to the surface of the gravel and become free-swimming. Because of the variation in the length of the incubation period and the differences in the depth at which the eggs are deposited, the young fish do not emerge suddenly all at once but come out from any nest in small groups when they have reached the required development.

The effect of temperature on the incubation period has already been shown by Kawajiri (1927) for a closely related species, *O. masou*, with the experimental demonstration that the number of days from fertilization to hatching decreases as the temperature rises. Although it is not possible to present similar definite proof for the pink salmon (*O. gorbuscha*) at McClinton creek, certain fairly sound circumstantial evidence indicates that the same condition exists.

Since there is a wide variation in point of time between the arrival of the first and last adult on the spawning areas, and between the instant of hatching of the first and last eggs, it is almost impossible to define exactly the boundaries between fertilization and egg rupture. A representative average for the time between fertilization and emergence can, however, be obtained by using as one limit the date at which half of the adult run had reached the spawning beds, and as the other, the date at which half of the fry had migrated to sea. Complete water temperatures are not available for all the years. It is known, however, that these water temperatures are directly correlated with the air temperatures in the region, which may, therefore, be substituted in such an analysis.

Table I gives the mean of the monthly average air temperatures taken at Masset for the autumn and winter (October to February) for the six years and the number of days between the limits specified for each run.

TABLE I. Mean of monthly average air temperature at Masset, B.C., during autumn and winter, and approximate time from fertilization to emergence of fry for pink salmon

Year	1930-1	1932-3	1934-5	1936-7	1938-9	1940-1
Air temp.						
(°C.)	6.1	4.4	5.0	5.0	7.8	7.8
(°F.)	43	40	41	41	46	46
Time (days)	225	247	244	231	227	207

While the gradation shown in the table is not exact, a condition not to be expected when such rough comparisons are employed, there is a definite indication that the period of time decreases with increase in temperature. As an example, in 1932-3 when the air temperature was approximately 40°F. (4.4°C.), the number of days was 247, while in 1938-9 and 1940-1 with air temperatures of 46°F. (7.8°C.) the elapsed times were 227 and 207 days respectively.

A difference of 40 days is the extreme for the six runs under consideration. The relatively small variation is very probably due to the fact that

air and thus stream temperatures are fairly constant in the autumn and winter months in the latitude in which the experiments were conducted.

Physical Characteristics of Free-swimming Fry

Pink salmon fry, when they become free-swimming, are uniform in size varying from 1.25 to 1.5 in. (3.2 to 3.8 cm.) in total length from the tip of the snout to the middle of the fork of the tail and weighing approximately 0.3 g. With few exceptions, they are at the same developmental stage. The yolk sac has been absorbed to the extent that there is little or no indication of it on the belly.

The young from McClinton creek, deep blue to bluish green on the top of the head and back with a clear unbroken silvery sheen on the belly, do not appear to differ in any particular from those taken at Cultus lake (Foerster and Pritchard 1935, p. 111). Albino individuals, though uncommon, do occur. These may be readily recognized by the absence of dorsal melanophores, which results in a distinctive pale yellow shade.

Counts of gill rakers, anal fin rays, dorsal fin rays, branchiostegal rays, and vertebrae made on samples from the various runs are not significantly different from those for young pinks of the same size at Cultus lake (Foerster and Pritchard 1935). No significant variation occurs from year to year. The following are the ranges and averages: gill rakers—12+5 to 15+6, 13+6; anal fin rays—14 to 17, 15; dorsal fin rays—12 to 15, 13; branchiostegal rays—8 to 13, 10; vertebrae—63 to 67, 64.

Downstream Migration

General Characters

The time occupied by the several yearly seaward migrations of fry varied from 92 to 125 days from the arrival of the first at the fence to the disappearance of the last. Usually only scattered individuals enter the pen during the first few days. The numbers gradually increase to large daily movements of fifty to one hundred thousand or more. Following this concentration the counts slowly fall off until the end of the run. The curve between time and numbers is, however, not absolutely smooth without irregularities. Daily variations do occur. As many as 700,000 have been handled in one twenty-four-hour period followed by only 235,000 in the next. The general trend, however, produces the slightly skewed normal frequency type illustrated by the counts for 1933 (fig. 4).

That there is no consistent relationship between the size of the fry runs and the time taken to leave the stream is shown by the following figures for each year: 1931—5,380,000, 123 days; 1933—2,230,000, 97; 1935—12,600,000, 125; 1937—3,675,000, 92; 1939—2,020,000, 103; and 1941—5,060,000, 107. Actually the largest migration amounting to approximately 12,600,000 fry did occupy the longest period, but the others, ranging in size from 2,020,000 to 5,380,000, maintained no special order.

The fry are not passively carried by the current out to sea, but actually make a swift and vigorous migration. They may move downstream as quickly as 15 to 20 ft. (4.6 to 6.1 m.), per sec., the only indication of their presence being given by the wake behind them. Halted temporarily at the fence, they may turn on their sides, but soon orientate themselves heads upstream and work along the screens to the pens. Such migratory activity is confined almost exclusively to the hours of darkness. As an example from a period chosen at random, from dusk to dawn on one night 146,000 fry entered the trap. The following day until dusk the next night only 4,000 were taken, and many of these might have been present in the morning and not removed when the count was made. During the next period of darkness, 152,000 appeared. In the daylight hours the young fish remain well hidden in the back-eddies, under roots and under stones.

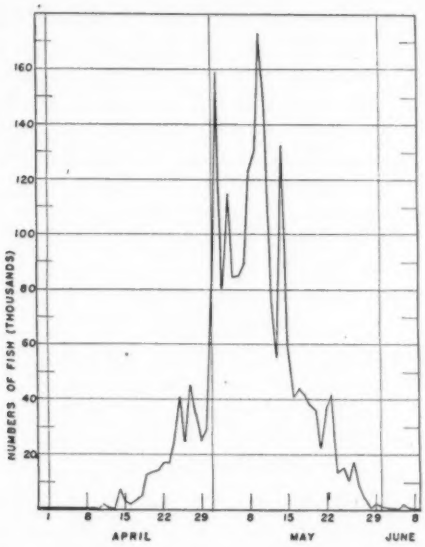


FIGURE 4. Frequency polygon for fry migration of 1933 at McClinton creek.

The course of the nightly migration was discovered by manipulating the pens in the trap. At a given time all the fish were driven into the downstream compartment. Those arriving during the remainder of the night collected in the upstream section. Repeated experiments of this type showed that under normal conditions the fry began running rather slowly, in so far as numbers were concerned, immediately after dusk. Very quickly they attained a constant rate which was maintained throughout the period of darkness. As dawn approached, the concentration decreased abruptly. If the percentages of the run which had occurred up to definite times were plotted against the percentages of the period between dawn and dusk which had been exhausted at the same times, a straight line at approximately

forty-five degrees to the horizontal (fig. 5) gave the best fit for the several points. Thus, within the accuracy of the experiments, the number of migrants per hour on any given night was constant. The slight delay in starting at dusk and the decrease at dawn apparently had little effect on the general trend.

Effect of Environmental Factors

LIGHT

That the migration is confined almost wholly to the hours of darkness and that during daylight the fry remain almost completely hidden in the back-eddies, under roots and under stones, has been reported under the description of normal conditions immediately above. The reaction may be even more specific. On occasion during the nightly migration period, bright moonlight prevailed. Actually, at such times, the moon illuminated only

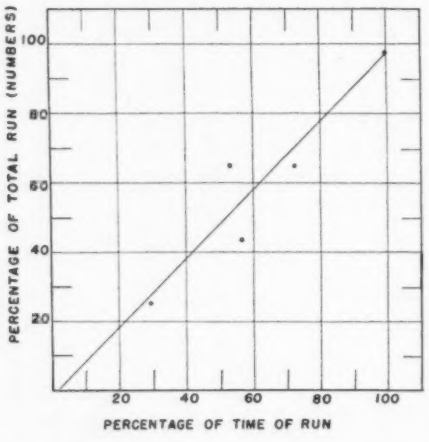


Figure 5. Course of nightly fry migration at McClinton creek.

parts of the winding river. When the rays did shine directly on the long stretch immediately above the fence, the migration slowed abruptly. Later when the area was exposed less directly to the moon's rays, the numbers again increased.

RAINFALL

The amount of rainfall during the autumn and winter months and its distribution in point of time has a noticeable effect on the numbers of fry which result from a given seeding. If during the spawning period sufficient water is available in the creek to allow the adult salmon to reach tne spawning areas and yet not enough to cover the higher gravel bars, the eggs will be deposited in the main creek channel from which the fry will have suitable conditions for egress at any time in the spring. If, at spawn-

ing, the water level is extremely high, the eggs will be deposited on the higher gravel bars and in small tributaries. Although there may be sufficient dampness at the depth of the egg-bearing stratum in such redds to permit incubation and hatching, the fry therein will have no possible means of reaching the main channel unless the water is again high at the time when they are sufficiently developed to migrate.

After deposition, the eggs are well protected by a covering of gravel 12 to 36 in. (0.3 to 0.9 m.) in depth. Even moderately large freshets, the result of heavy rainfall, will have little effect. On occasion, however, extreme conditions do arise. Pritchard (1938, p. 147) records a large snowfall in November, 1933, followed by 21.36 in. (54.3 cm.) of rain of which over 4 in. (10 cm.) were concentrated in the last two days of December and the first five in January. The resulting freshet was disastrous, tearing up the gravel beds and washing out the eggs. Only five fry were obtained from a planting of 540,294 eyed eggs.

The influence of rainfall and the resultant rise in water height on the daily migration is best indicated by the recoveries from the "threading" experiments. Under conditions of low water the marked individuals reached the fence during darkness on succeeding nights over a period of from four to nine days after release upstream. It was apparent that the nearer the point of release to the trap, the more quickly was the migration accomplished. When a freshet occurred which maintained the water at a high level during the night, the fish moved more quickly. In the experiments under high water conditions, recoveries at the counting weir were made in one to three days. If the freshet took place in the daytime, there was no sudden influx of fry into the pens. Evidently the young fish did not immediately come out from under the roots, etc., and hasten toward the river mouth. Thus rainfall and the consequent rise in water would not appear to be the dominant stimulus. In this particular case light seems more important, otherwise migration would have been equally as heavy in the daytime.

One explanation of the reaction of the fish under freshet conditions can be seen in their usual behaviour. During daylight hours the fry are not swimming freely around but are hidden under stones and any other projection such as roots. During darkness they leave their hiding places and rise to the surface. With the increase in the size of the stream and the current, those which would ordinarily be in calm water are swept into the main flow whence they carry on their migration toward the sea. On the night of the freshet most of the young fish which are out of the gravel move downstream. Under conditions of low water, many, perhaps even the majority, do not migrate but return to shelter and may not run until some nights later. This hypothesis is supported by the observation, many times confirmed, that for the first hours of a freshet the numbers migrating are larger than normal. Later, even though the stream may be just as high, fewer appear. This sudden decrease is taken as indicating that the availability of the fish out of the gravel is becoming reduced.

TEMPERATURE

The possibility that the temperature has a definite effect on the length of time between deposition of pink salmon eggs and the emergence of the fry from the gravel has been discussed above at some length. Within any twenty-four hour period it is impossible to demonstrate conclusively any particular influence. Usually the variations in water temperature each day are small. When the temperatures appear to be almost similar on succeeding days, the changes in the counts are so large that they almost completely mask any effect which might be directly attributable to the factor.

OTHER ENVIRONMENTAL FACTORS

There are other environmental factors which vary coincidentally with light, rainfall and temperature. Ordinarily during the daytime, the oxygen content of the water is high and the carbon dioxide content low. At night the opposite situation prevails. Analyses have shown that during freshets the daily fluctuation in oxygen content is not so marked. There are some slight but inconsistent changes in the pH. Undoubtedly there is the possibility that the changes in any one of these or other factors not mentioned might be the cause for the beginning or end of the daily migration. The variations are so small, however, as to appear insufficient to cause such a sudden change in activity. In addition the changes take place very gradually and in the main not at the time when the migration commences or stops.

SUMMARY

Hatching of pink salmon from the eggs takes place two to three and one-half months after fertilization. The yolk-sac fry may remain in the redds as long as three and one-half months before emergence. High temperatures appear to shorten the length of the time between egg deposition and fry emergence.

The fry from McClinton creek are similar to those previously described from Cultus lake (Foerster and Pritchard 1935). There is no significant variation either in the qualitative description or in the counts of gill rakers, anal and dorsal fin rays, branchiostegal rays and vertebrae from year to year.

The yearly seaward migration may occupy from 92 to 125 days beginning with a few scattered individuals, reaching a peak of fifty to one hundred thousand or more per day, and dropping off quickly. There is no relationship between the size of the run and the time it takes to get to sea.

The fry actually make a swift and vigorous migration moving downstream as quickly as 15 to 20 ft. (4.6 to 6.1 m.) per sec. This migration begins rather slowly at dusk each day, quickly reaches a constant rate, in so far as numbers are concerned, which is maintained until dawn. Relatively few move during daylight.

The effect of light is evident from the fact that the activity is confined to the hours of darkness and that the moon's rays over a long area of the

river will slow the movement. Rainfall may affect the numbers which result from a given seeding. It will cause the fry to reach sea more quickly if they are already migrating but it apparently does not start migration. Temperature variations are so small in most cases that they do not seem responsible for the major changes in behaviour noted. The effect of other variables such as the oxygen content of the water, the carbon dioxide content, and the pH, cannot be established.

ACKNOWLEDGEMENTS

During the latter half of the investigation, 1938 to 1941, the writer was assisted by Mr. W. M. Cameron. He is mainly responsible for the results obtained from the "threading" experiments. The various members of the staff of the Pacific Biological Station have been particularly helpful with advice and criticism both during the experiment and in the preparation of the manuscript.

REFERENCES

FOERSTER, R. E. *Contr. Canad. Biol. Fish.*, **5** (3), 57-82, 1929.
FOERSTER, R. E., AND A. L. PRITCHARD. *Rep. B.C. Comm. Fish.*, **1934**, 106-116, 1935.
KAWAJIRI, MINORU. *J. Imp. Fish. Inst. Japan*, **23** (2), 14-21, 1927.
PRITCHARD, A. L. *J. Fish. Res. Bd. Can.*, **4** (2), 141-150, 1938.

Sexual Dimorphism in the Lobster (*Homarus americanus*)

By Wilfred Templeman
Memorial University College
St. John's, Newfoundland

(*Received for publication November 9, 1943*)

ABSTRACT

In relation to total length of body, the longer carapace and the accessory copulatory appendage of the male and the longer fourth swimmeret of the female are seen in the immature but develop further during and after sexual maturity. The small sternal spines and long second swimmeret of the female and the wider carapace of the male develop with sexual maturity and become more pronounced with increase in body size.

The data on which this article is based were obtained while the author was employed at the Atlantic Biological Station, St. Andrews, New Brunswick, and at the Fisheries Research Laboratory of Newfoundland. Relative sizes of the different parts are all based upon the animal's total length measured from the tip of the rostrum to the end of the telson, with the lobster alive and lying on its back. Only lobsters with imperfect rostra were rejected. Hairs or setae were excluded in all measurements.

It was found (Templeman 1939) that the *relative length of the carapace* in the male increases sharply when the lobsters are becoming sexually mature. This size varies with locality and the relative increase varies accordingly (fig. 1). While this relative length seems to remain fairly steady before sexual maturity it continues to increase with size afterwards even to the largest measured.

At all commercial sizes the relative length of the carapace is less in the female than in the male. It remains constant when the lobsters are small, but shows a definite and progressive increase for those localities for which large lobsters were available for measurement (fig. 1).

It is not certain that the small differences to be seen (horizontal portions of the graphs, fig. 1) in the relative length of the carapace in immature males and females from different localities represent local differences, since the numbers of lobsters measured were not very great, the condition of the lobsters which affects total length measurements may have varied, and measurements were made by different methods (A, C, E and F by dividers, and B and D by calipers). It might be expected, however, that since this relative length remains relatively constant in the immature, it would be the same from place to place.

The ratio of the *greatest width of the carapace* to total length (fig. 2) is in the

J. Fish. Res. Bd. Can 6 (3) 1944
Printed in Canada.

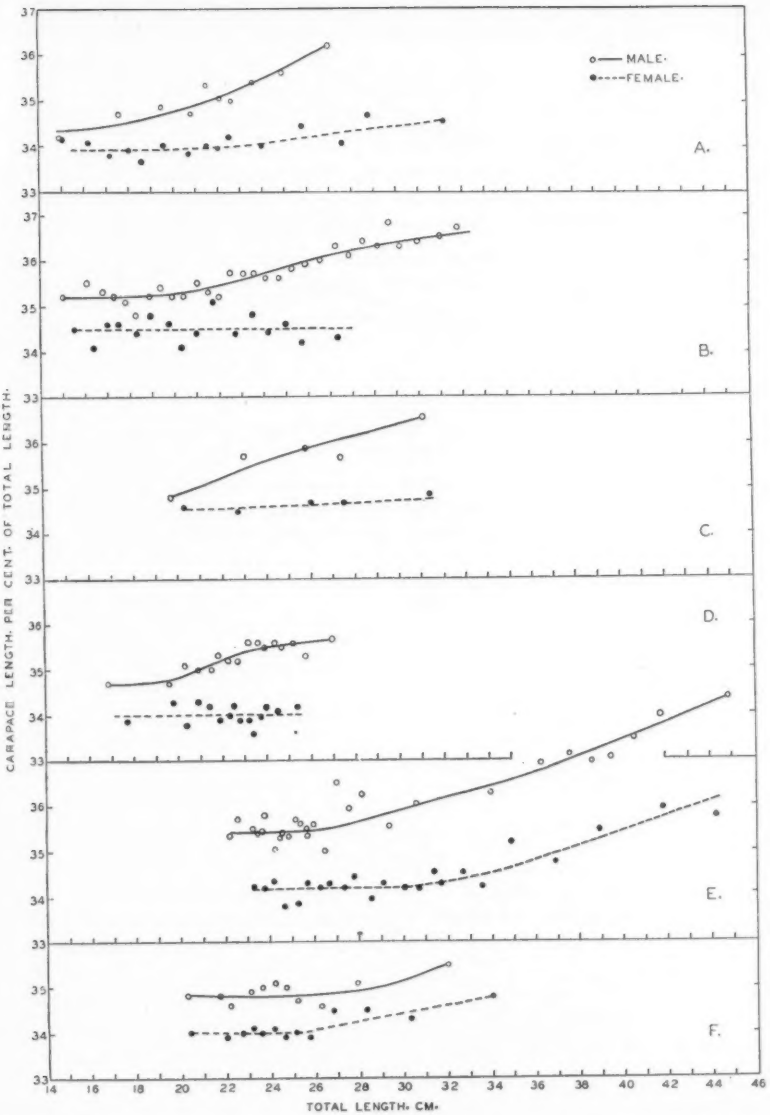


FIGURE 1. Relation of carapace length, from the bottom of the eye socket directly back, to the total length for 5-specimen (A, E) or 10-specimen (B, C, D, F) lots of male and female lobsters. A, Pte. du Chêne, 1937; B, House Hr. lagoon, Magdalen is., 1931; C, Canso, 1932; D, Port au Port, St. George's bay, 1938; E, Placentia, 1939; F, Seal Cove, Grand Manan, 1932.

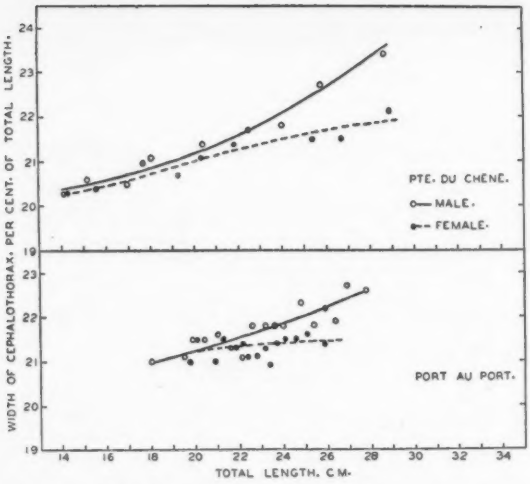


FIGURE 2. Relation of cephalothorax width to total length for 5-specimen lots of male and female lobsters at Pte. du Chêne, 1936, and Port au Port, St. George's bay, Newfoundland, 1938. Carapace width measured between second and third pereiopods.

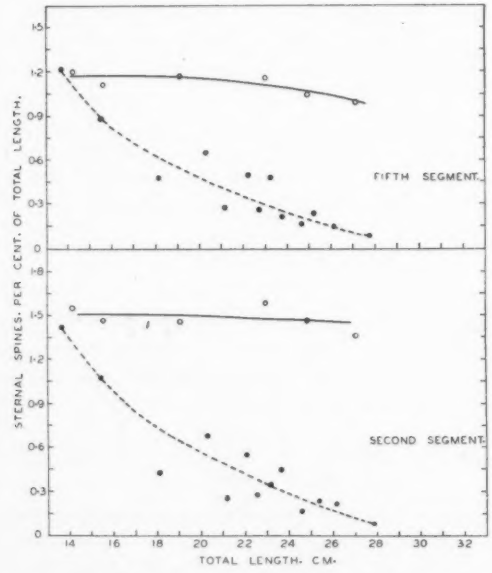


FIGURE 3. Relation between the length of the sternal spines on the second and fifth segments of the abdomen, to the total length for 5-specimen lots of male and female lobsters at Pte. du Chêne, 1936. Male,—clear circles, solid line; female,—solid circles, broken line.

smaller relatively immature lobsters approximately the same for both males and females, while in the larger animals it increases with size and more so in the male than in the female.

There is a gradual decrease in the length of the single *spines on the sterna of the abdomen* from the 2nd to the 5th (none on the 1st and 6th), except that in the male the spines on the 2nd and 3rd are equal. (Pte. du Chêne, N.B., in 8 males of 17.9 cm. average length, the spines were 1.41, 1.41, 1.28 and 1.1% respectively of the total length and in 11 females of 18.2 cm. average length, the spines were 0.65, 0.59, 0.57 and 0.53% of the total length.) The ratio of spine length to total length decreases only slightly with increasing size in the male, but decreases sharply in the female as sexual maturity approaches (fig. 3). In

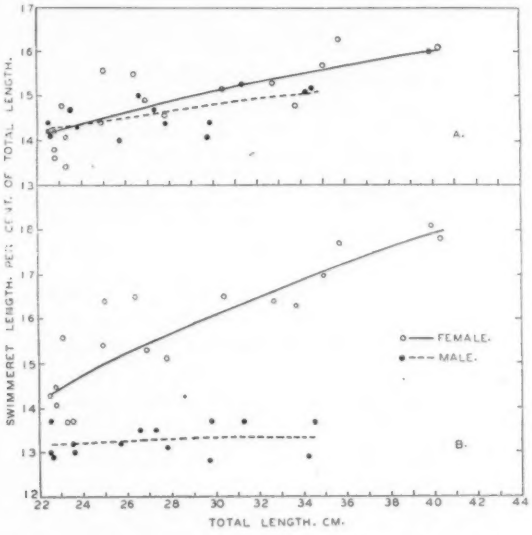


FIGURE 4. Relation between the length of the second (A) and fourth swimmerets (B) and the total length for single specimens of male and female lobsters in Placentia bay, Newfoundland, 1939.

mature egg-bearing females the spines are usually scarcely visible blunt knobs, which will not puncture the eggs when the abdomen is closed quickly.

In the male the *swimmerets* (including protopodite and endopodite) on the second abdominal segment are the longest and in the female those on the third and fourth are the longest and approximately equal. The second swimmerets (fig. 4A) are not greatly different in length in males and females, but they do increase more rapidly in length in the female. The fourth swimmerets (fig. 4B) on the contrary remain relatively constant in relation to total length in the male, but increase rapidly in relative length in the female, which permits a greatly increased number of eggs to be carried where there is more room with the abdomen normally somewhat flexed.

The *accessory copulatory appendage* of the male (on the swimmeret of the 2nd abdominal segment), as measured from base posteriorly to tip, rapidly increases in relative size with approach of sexual maturity and continues this relative increase more slowly thereafter (fig. 5).

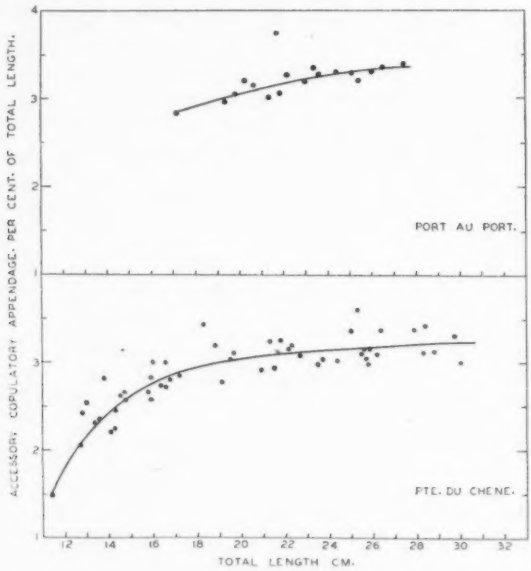


FIGURE 5. Relation in the male lobster between the length of the accessory copulatory appendage on the second swimmeret and the total length for 5-specimen lots at Port au Port, 1938, and for single specimens at Pte. du Chêne, 1936.

REFERENCE

TEMPLEMAN, W. *Dept. Nat. Res. Nfld. Res. Bull.* **7** (*Fish.*) 1-52, 1939.

Action of Nitrates and Nitrites on Bacteria

By H. L. A. Tarr
Pacific Fisheries Experimental Station

(*Received for publication October 26, 1943*)

ABSTRACT

The amount of $NaNO_2$ formed by reduction of $NaNO_3$ by growing heterotrophic fish spoilage bacteria depended largely on the pH of the culture medium used. Thus $NaNO_2$ formation was strongly inhibited in media controlled at acid pH, but amounts as high as 6,950 p.p.m. were formed when the pH was permitted to rise unchecked $NaNO_3$, in presence or absence of 200 p.p.m. of $NaNO_2$, had either a very trivial effect, or no effect, on the growth of fish spoilage bacteria in broth medium or fish muscle, and it was either not, or but feebly, reduced in fish muscle under the experimental conditions.

In previous papers (Tarr 1941, 1942) the bacteriostatic and bactericidal action of sodium nitrite was studied in detail with special reference to the effect of pH. Sodium nitrate is generally conceded to have practically no direct bacteriostatic action, other than that which might be due to osmotic effects when large concentrations of the salt are used (Holm and Sherman 1921). However, nitrates may influence bacterial growth in two different ways. They may be activated by bacterial *nitratase* and act as hydrogen acceptors in place of molecular oxygen, thereby rendering anaerobic growth possible, or at least facilitating it (Quastel, Stephenson, and Whetham 1925). On the other hand they have been shown to inhibit growth of, and toxin formation in, certain *Clostridia* (Tanner and Evans 1933). Nitrates inhibit growth of *Clostridia* in meat-curing processes. This is believed to be due to an indirect effect in which the hydroxylamine formed during bacterial reduction of nitrates inactivates catalase, thereby permitting an accumulation of hydrogen peroxide which is extremely toxic for *Clostridia* even in small concentrations (Jensen 1942); hydroxylamine has been shown to be formed during the reduction of nitrates by certain facultative anaerobes (Lindsey and Rhines 1932), and is probably similarly formed by certain *Clostridia* (Woods 1938). It has been shown that nitrites inhibit the growth of certain species of *Clostridia* in acid medium (Tarr 1942), but whether this finding applies to the nitrites formed by bacterial reduction of nitrates in meat curing processes is apparently not known.

The present work was undertaken in order to determine (1) the optimum conditions for production of nitrites from nitrates by fish spoilage bacteria, and

J. Fish. Res. Bd. Can. 6 (3) 1944.
Printed in Canada

(2) the effect of nitrates and mixtures of nitrates and nitrites on growth of such organisms in laboratory media at different pH values and in fish muscle itself.

EXPERIMENTAL

Reduction of Nitrates at Different pH Values

Though the reduction of nitrates to nitrites is one of the principal tests used in classifying bacteria, little appears to be known regarding the amount of nitrite which is produced under different conditions. Stickland (1931), when studying the *nitratase* enzyme of *B. coli*, found that washed suspensions of this organism produced in one instance 2,480 p.p.m. of potassium nitrite from 2,520 p.p.m. of the corresponding nitrate. The following experiments show that comparatively large amounts of nitrite can be formed from nitrate by growing fish spoilage bacteria under certain conditions. No attempt has been made to determine how much nitrite can be formed from the corresponding nitrate by washed suspensions of such organisms. The cultures employed were those used in previous experiments (Tarr 1939).

EXPERIMENT 1.

Culture 7 (*Micrococcus* sp.) and culture 23 (*Achromobacter* sp.), the growth of both of which had been shown in previous experiments to be strongly inhibited by 0.02 per cent sodium nitrite, were cultivated 24 hours on Bacto nutrient agar. A small amount of the growth from each culture was transferred to each of two 500-ml. conical flasks containing 250 ml. of Bacto nutrient broth plus 1 per cent chemically pure $NaNO_3$ (pH 7.0). The flasks were incubated at 25° C. and at stated intervals small portions were removed aseptically and the following three determinations were made: (1) pH to the nearest 0.05 unit using a Beckman pH meter; (2) direct bacterial counts using a Petroff-Hausser counting chamber; and (3) amount of $NaNO_2$ using an Evelyn photo-electric colorimeter and a No. 520 Rubicon filter (White 1939). The comparatively low bacterial counts obtained with culture 7 were due to the fact that this organism formed clumps in liquid medium, and that these were recorded as a single unit.

The results (table I) showed that in nutrient broth at an initial pH of 7.0 the two cultures produced rather similar amounts of $NaNO_2$ from 10,000 p.p.m. of $NaNO_3$, maximum production (4,050 and 4,250 p.p.m.) being reached after 27 to 39 days' incubation. The production of $NaNO_2$ was accompanied by a pronounced increase in bacterial content up to the eleventh or seventeenth day and by a marked increase in pH.

EXPERIMENT 2.

Bacto nutrient broth containing 1 per cent $NaNO_3$ and 0.05 M KH_2PO_4 was prepared and adjusted to pH 5.95 with 1.0 N NaOH. Analytical reagent grade glucose was added in 0.1 per cent concentration to a portion of this medium. The resulting media were dispersed in 250-ml. portions into 500-ml. conical flasks, which were then autoclaved, the pH rising to 6.00.

Four flasks of the plain broth and two of the glucose broth were inoculated

TABLE I. Amount of $NaNO_2$ formed from $NaNO_3$ in Bacto nutrient broth at an initial pH of 7.0 in duplicate experiments (A and B).

Incubation period at 25°C. (days)	$NaNO_2$ (p.p.m.)				pH				Bacterial counts ($\times 10^6$ per ml.)			
	Culture 7		Culture 23		Culture 7		Culture 23		*Culture 7		Culture 23	
	A	B	A	B	A	B	A	B	A	B	A	B
0	0.2	0.2	0.2	0.2	7.00	7.00	7.00	7.00	..	..	...	...
3	1500	1800	750	790	6.80	6.80	7.00	7.00	25	26	440	415
11	3050	2850	2650	2700	7.85	7.85	7.90	7.85	51	52	580	550
17	3800	3600	3250	3450	8.30	8.20	8.35	8.30	76	82	**	**
27	3900	3850	4050	3950	8.50	8.40	8.75	8.70	81	85	**	**
39	4100	4250	4000	3900	8.55	8.60	8.90	8.80	79	95	**	**
45	3650	4250	4050	3850	8.55	8.60	8.85	8.80	78	89	**	**

*This organism forms clumps and the counts given represent clumps or single organisms.
**Cells autolyzing and accurate count impossible.

with 0.5 ml. of a young broth culture of organisms 7 and 23 (*Achromobacter* sp.), the inocula containing 1.4×10^6 cells (mostly clumps) and 85×10^6 cells respectively. The inoculated media were incubated at 25° C., and were examined at stated intervals as described in experiment 1. In the case of each organism the pH of the medium in both duplicates of one of the two pairs of broth cultures was kept at about 6.0 by occasional careful addition of sterile 1.0 N HCl, the pH in the remaining pair being permitted to rise unchecked. After the $NaNO_2$ content of the various cultures had risen to between 200 and 300 p.p.m., 10 ml. of toluene was added to one of each pair, the flasks being then stoppered and shaken well.

The results of this experiment are given in tables II and III. It was found that in all instances toluene treatment promptly stopped bacterial increase and soon suppressed $NaNO_2$ formation, there being some increase of the latter during the day following such treatment but none thereafter. In the cultures in which no attempt was made to hinder the normal increase in pH the $NaNO_2$ gradually increased until it reached 6,950 p.p.m. after 65 days with production of a pH of 8.55 in culture 23, and 1,800 p.p.m. after 76 days with production of a pH of 6.50 in culture 7. On the other hand nitrite formation was markedly suppressed when the normal increase in pH was checked either by addition of HCl or by acid formed from glucose. Thus in culture 7 the pH of the glucose medium soon fell to pH 5.7, and the $NaNO_2$ content did not rise above 285 p.p.m.; while in culture 23 the pH fell to 5.65 in this medium and the $NaNO_2$ content did not rise above 420 p.p.m. When the pH of the ordinary buffered broth medium was not allowed to rise above 6.00 due to occasional addition of HCl, the $NaNO_2$ content did not rise above 880 p.p.m. in culture 7 and 780 p.p.m. in culture 23. The bacterial counts were complicated by the fact that the organisms in culture 7 formed clumps throughout the experiment, while in culture 23 clumps of curious coccoid involution forms appeared after about two weeks. Autolysis also

TABLE II. Amount of $NaNO_2$ formed from $NaNO_3$ in duplicate experiments (A and B) with culture 7 (*Micrococcus* sp.) in Bacto nutrient broth containing 1 per cent $NaNO_3$ (initial pH 6.00) with pH adjusted, pH not adjusted and with 0.1 per cent glucose. Inoculum 1.4×10^6 cells or clumps of cells per flask.

Incubation period at 25°C. (days)	$NaNO_2$ (p.p.m.)						pH						Bacterial counts ($\times 10^6$ per ml.)					
	pH not adjusted		pH adjusted		0.1% glucose		pH not adjusted		pH adjusted		0.1% glucose		pH not adjusted		pH adjusted		0.1% glucose	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
0	0.2	0.2	0.2	0.2	0.2	0.2	6.00	6.00	6.00	6.00	6.00	6.00	..	..	..	..	..	..
2	..	..	..	..	*235	215	..	..	..	..	*5.70	5.70	..	..	..	..	*0.60	0.55
3	250*	230	*210	260	255	285	*6.00	6.00	*6.00	6.00	5.70	5.70	*0.55	0.65	*0.45	0.60	0.65	0.60
5	240	465	225	420	250	285	6.00	6.05	6.00	6.00	5.70	5.70	0.55	1.00	0.45	1.30	0.55	0.50
7	240	560	220	700	240	280	6.00	6.10	6.00	6.00	5.70	5.70	0.55	3.90	0.45	4.1	0.60	0.50
10	..	1500	..	860	..	285	..	6.25	..	6.20	..	5.70	..	63	..	30	..	5.2
12	..	1490	..	880	..	285	..	6.30	..	6.00	..	5.70	..	174	..	32	..	7.0
16‡‡	..	1500	..	830	..	275	..	6.30	..	6.00	..	5.70	..	180	..	18	..	9.0
20	..	1550	..	..	..	260	6.00	6.30	6.00	6.00	5.70	5.70	0.88	185	0.7	12	0.80	0.7
35	..	1400	..	..	..	..	..	6.35	..	..	..	..	..	250	..	..	..	..
42	..	1400	..	..	..	..	..	6.40	..	..	..	..	..	328	..	..	..	..
55	..	1750	..	..	..	..	..	6.50	..	..	..	..	..	..	..	..	..	..
64	..	1750	..	..	..	..	..	6.50	..	..	..	..	..	..	..	..	..	..
76	..	1800	..	..	..	..	..	6.50	..	..	..	..	..	‡243	..	..	..	..
82	..	1400	..	..	..	..	..	6.50	..	..	..	..	..	..	..	..	..	..

*Toluene added.

‡Autolyzing cells made an accurate count impossible.

‡‡All except "pH not adjusted, B" contained no viable bacteria in 1 ml. of medium.

TABLE III. Amount of $NaNO_2$ formed from $NaNO_3$ in duplicate experiments (A and B) with culture 23 (*Achromobacter* sp.) in Bacto nutrient broth containing 1 per cent $NaNO_3$ (initial pH 6.00) with pH adjusted, pH not adjusted and with 0.1 per cent glucose. Inoculum 85×10^6 cells per flask.

Incubation period at 25°C. (days)	$NaNO_2$ (p.p.m.)						pH						Bacterial counts ($\times 10^6$ per ml.)					
	pH not adjusted		pH adjusted		0.1% glucose		pH not adjusted		pH adjusted		0.1% glucose		pH not adjusted		pH adjusted		0.1% glucose	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
0	0.2	0.2	0.2	0.2	0.2	0.2	6.00	6.00	6.00	6.00	6.00	6.00	..	..	..	..	..	..
1	95	90	105	110	90	80	6.05	6.05	6.00	6.00	5.70	5.70	72	78	68	71	142	194
2*	285	280	280	300	265	280	6.05	6.05	6.00	6.00	5.65	5.65	87	83	78	89	139	191
3	295	500	310	530	320	420	6.05	6.10	6.00	6.05	5.65	5.65	93	88	73	86	142	187
4	290	610	310	560	310	405	6.05	6.15	6.00	6.05	5.65	5.65	88	84	73	93	138	181
6	285	735	320	780	310	400	6.05	6.20	6.00	6.00	5.65	5.65	91	88	79	84	134	184
8	280	720	310	770	310	400	6.05	6.20	6.00	6.00	5.65	5.65	..	..	..	..	..	..
10	280	720	315	755	310	410	6.05	6.20	6.00	6.00	5.65	5.65	87	83	74	85	139	179
13‡‡	..	1140	..	..	..	..	..	6.30	..	..	..	..	..	‡	..	..	..	..
17	280	1950	..	..	..	..	6.05	6.50	..	..	..	..	82	..	..	..	..	..
20	285	2500	..	..	..	..	6.05	6.60	..	..	..	..	..	..	..	..	..	..
27	260	3100	..	..	..	..	6.05	6.75	..	..	..	..	..	..	..	..	..	..
36	215	3450	..	..	..	..	6.05	7.15	..	..	..	..	..	..	..	..	..	..
43	215	4850	..	..	..	..	6.05	7.65	..	..	..	..	..	..	..	..	..	..
56	..	5750	..	..	..	..	..	8.40	..	..	..	..	..	..	..	..	..	..
65	..	6950	..	..	..	..	..	8.55	..	..	..	..	..	..	..	..	..	..
77	..	6750	..	..	..	..	..	8.60	..	..	..	..	..	..	..	..	..	..
83	170	5900	..	..	..	..	6.05	8.60	..	..	..	..	..	..	..	..	..	..

*Toluene added to all "A" cultures.

‡Cells clumping and autolyzing.

‡‡All except "pH not adjusted, B" contained no viable bacteria.

contributed to the difficulty of making accurate counts. There was a general tendency for $NaNO_2$ increase to be accompanied by bacterial increase in both cultures, but the above mentioned factors made any close comparison of this relation impossible. There was not necessarily any direct relation between bacterial content and concentration of $NaNO_2$ formed, for in culture 23 glucose broth cultures had more bacteria but considerably less $NaNO_2$ than had the corresponding plain broth cultures up to the tenth day. That the $NaNO_2$ produced was quite toxic at acid pH values was suggested by the fact that the glucose-containing and pH-adjusted cultures of both organisms contained no viable bacteria after 13 to 16 days' incubation. On the other hand the cultures in which the pH was not controlled contained viable organisms 105 days after commencement of the experiment.

EXPERIMENT 3

An experiment closely similar to the above was carried out using culture 23 and, in place of Bacto nutrient broth, a fish digest broth medium (Tarr 1942) having an initial pH of 5.90. In this experiment duplicates were carried throughout, no toluene being added. The results (table IV) showed that the duplicates agreed fairly closely, but were somewhat different from those of the foregoing experiment. Thus in the glucose medium the initial fall in pH was purely transient, the organism evidently resisting the amount of $NaNO_2$ formed at this pH. After 70 days the pH averaged 8.2 and the $NaNO_2$ greatly increased, though not so markedly as in the digest containing no glucose. In the cultures in which the pH was controlled by addition of HCl, the $NaNO_2$ content never rose above 375 p.p.m.; while in those in which it was permitted to rise unchecked to pH 8.25 or 8.3, from 3,800 to 4,450 p.p.m. of $NaNO_2$ were formed.

TABLE IV. Amount of $NaNO_2$ formed from $NaNO_3$ in duplicate experiments (A and B) with culture 23 (*Achromobacter* sp.) in fish digest broth containing 1 per cent $NaNO_3$ (initial pH 5.90) with pH adjusted, pH not adjusted and with 0.1 per cent glucose. Inoculum 36×10^6 cells per flask.

Incubation period at 25°C. (days)	$NaNO_2$ (p.p.m.)						pH						Bacterial coupts ($\times 10^6$ per ml.)					
	pH not adjusted		pH adjusted		0.1% glucose		pH not adjusted		pH adjusted		0.1% glucose		pH not adjusted		pH adjusted		0.1% glucose	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
4	180	155	54	130	33	32	6.00	6.00	5.95	5.95	5.90	5.95	21	19	19	20	28	31
5	265	245	130	145	150	155	6.10	6.10	5.95	5.95	5.80	5.80	20	23	24	20	28	30
7	485	440	285	275	265	270	6.20	6.20	6.00	6.00	5.85	5.85	19	30	22	32	32	44
10	700	695	375	370	255	255	6.50	6.50	6.00	6.00	6.00	6.00	35	36	32	32	47	51
18	800	790	305	315	365	380	6.60	6.60	6.00	6.00	6.30	6.30	88	83	34	31	50	50
26	1300	1300	315	335	740	725	6.90	6.90	6.10	6.10	6.50	6.50	145	138	40	44	84	74
33	2150	2550	275	290	1650	1250	7.40	7.30	6.00	5.95	7.00	6.75	940	812	44	43	792	660
45	3350	3500	280	300	2400	2150	8.05	7.90	6.00	6.00	7.85	7.40	*	*	*	*	*	*
54	3200	3700	270	290	2750	2500	8.30	8.15	6.00	6.00	8.15	7.90						
64	3650	4450	270	285	3200	3600	8.30	8.25	6.00	6.00	8.25	8.10						
70	3800	4200	265	280	2850	2800	8.30	8.25	6.00	6.00	8.25	8.15						

*Autolyzing cells and formation of clumps of coccoid forms made further accurate counts impossible.

Influence on Growth in Culture Media

In order to ascertain whether nitrate itself inhibited growth, or whether the inhibition, if any, depended on prior reduction of nitrate to nitrite, half the

Table V. Effect of $NaNO_3$, $NaNO_2$, and a mixture of these salts on the growth (−means "none, + t "some

*Culture	Inoculum **(× 10^6)	Initial pH of medium†	Amount of growth								
			Control			1% $NaNO_2$			0.5% $NaNO_3$		0.1%
			1 day	2 days	14 days	1 day	2 days	14 days	1 day	2 days	14 day
7		5.95	−	+	+++	+	++	++	+	+	++
Micrococcus	1.6	6.35	+	++	+++	+	++	++	++	++	+++
+		7.20	++	++	+++	++	++	+++	++	++	+++
18		5.95	−	−	−	−	+	−	+	+	−
Micrococcus	1.3	6.35	+	+	−	−	+	−	+	+	−
+		7.20	+	+	+	−	+	−	+	+	−
22		5.95	+++	+++	+++	++	++	+++	++	++	+++
Achromobacter	6.8	6.35	++	+++	+++	++	+++	+++	++	+++	+++
+		7.20	++	+++	+++	+++	+++	+++	+++	+++	+++
23		5.95	++	++	+++	++	++	+	++	++	++
Achromobacter	4.5	6.35	++	++	+++	++	++	++	++	++	+++
+		7.20	++	++	+++	++	+++	+++	++	+++	+++
9		5.95	++	++	+++	++	++	+++	++	++	+++
Achromobacter	1.9	6.35	++	++	+++	++	++	+++	++	++	+++
−		7.20	++	+++	+++	++	++	+++	++	++	+++
19		5.95	++	+++	+++	++	+++	+++	++	+++	+++
Achromobacter	6.1	6.35	++	+++	+++	++	+++	+++	++	+++	+++
−		7.20	++	+++	+++	++	+++	+++	++	+++	+++
10		5.95	+	+	−	+	+	−	+	+	−
Micrococcus	1.8	6.35	+	+	−	+	+	−	+	+	−
−		7.20	++	++	+++	++	++	+++	++	++	+++
24		5.95	−	−	++	−	−	++	−	−	++
Micrococcus	4.7	6.35	+	++	+++	+	++	+++	+	++	+++
−		7.20	++	+++	+++	++	+++	+++	++	+++	+++

*In this column + indicates that a culture reduced nitrate to nitrite strongly, − that it did not reduce
**All the *Micrococcus* formed clumps in broth and the counts given represent clumps plus single cells.
†These pH values were approximately 0.15 of a unit lower in media containing 1% $NaNO_3$, and 0.1 of a

cultures chosen were ones which reduced nitrate to nitrite strongly, while the remainder did not.

Sterile 16 × 150-ml. tubes received 2 ml. of double strength Bacto nutrient broth; 0.5 ml. of one of three 0.2 M phosphate buffers (pH 4.4, 6.0, or 7.5); 1 ml. of a sterile solution of $NaNO_3$, $NaNO_2$, a mixture of these, or water, and

one, + to "some" to "much") of certain fish spoilage bacteria in Bacto nutrient broth of different pH values.

	Amount of growth														
NaNO₃	0.1% $NaNO_3$			0.02% $NaNO_2$			1% $NaNO_3$ + 0.02% $NaNO_2$			0.5% $NaNO_3$ + 0.02% $NaNO_2$			0.1% $NaNO_3$ + 0.02% $NaNO_2$		
rs	1 day	2 days	14 days	1 day	2 days	14 days	1 day	2 days	14 days	1 day	2 days	14 days	1 day	2 days	14 days
	+	+	++	−	−	++	−	−	−	−	−	+	−	−	++
+	++	++	+++	+	+	++	+	++	++	+	+	++	+	+	++
−	++	++	+++	++	++	+++	++	++	+++	++	++	+++	++	++	+++
	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	−	−	−	+	+	−	+	++	+++	−	−	+++	−	+	−
	++	++	+++	+	++	+++	+	+	++	+	+	++	+	+	+++
+	++	+++	+++	++	+++	+++	++	++	+++	++	++	+++	++	++	+++
−	++	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−
	+	++	++	+	++	+++	−	+	++	−	+	+	+	+	+
−	++	+++	+++	++	++	+++	++	++	+++	++	++	+++	++	++	+++
	++	++	+++	−	−	−	−	−	−	−	−	−	−	−	−
	++	++	+++	−	−	−	−	−	+++	−	+	+++	−	−	+++
	++	+++	+++	++	+++	+++	++	++	+++	++	+++	+++	++	+++	+++
	++	+++	+++	−	−	−	−	−	−	−	−	−	−	−	−
	++	+++	+++	+	++	+++	+	++	+++	+	++	+++	+	++	+++
	++	+++	+++	++	+++	+++	++	+++	+++	++	+++	+++	++	+++	+++
	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
	−	+	−	+	+	−	−	+	−	+	+	−	−	+	−
	++	++	+++	++	++	+++	++	++	+++	++	++	+++	++	++	+++
	++	−	++	−	−	−	−	−	−	−	−	−	−	−	−
	++	++	+++	+	++	+++	−	−	+++	−	++	+++	+	++	+++
	++	+++	+++	++	+++	+++	++	+++	+++	++	+++	+++	++	+++	+++

educe trate to nitrite.

ls.

l of a mit lower in media containing 0.5% $NaNO_3$.

0.5 ml. of an inoculum prepared by suspending a portion of a 24-hour broth culture of the organism under investigation in sterile 0.1 per cent NaCl. In

this manner a number of media of different pH values and containing nitrate, nitrite, or nitrate plus nitrite in varying proportions were prepared (table V). It was found that 1 per cent $NaNO_3$ lowered the initial pH of the broth by approximately 0.15 of a pH unit, while 0.5 per cent lowered it approximately 0.1 of a pH unit. The inoculated tubes were incubated at 25°C. and examined for visible growth at stated intervals.

It was found (table V) that the effect of $NaNO_3$ alone was both slight and inconsistent, and did not appear to be influenced by whether a given culture did or did not reduce nitrate to nitrite. Thus at acid pH it suppressed growth slightly in the case of culture 23, but under similar conditions favoured the growth of culture 18. In the other cultures nitrate had little or no effect on growth. As in previous work (Tarr 1941, 1942) 0.02 per cent sodium nitrite alone exerted a more or less pronounced inhibition of growth of all cultures studied at acid pH. In the case of culture 9 a mixture of $NaNO_3$ and $NaNO_2$ appeared to be less effective in inhibiting growth than was $NaNO_2$ alone, while in culture 24 both 1 and 0.5 per cent $NaNO_3$ stimulated the bacteriostatic action of the $NaNO_2$ slightly. Poor growth and rapid autolysis occurred in most of the media inoculated with culture 23, the same holding true in the case of culture 10 in media of acid pH.

Influence on Growth in Fish Muscle

Strictly fresh fish were filleted under clean conditions, the flesh was passed through a domestic mincer, and, after mixing thoroughly, 95-g. portions were transferred to sterile 250-ml. beakers covered with an inverted petri dish. The desired quantity of $NaNO_2$, $NaNO_3$ or NaCl was added in a 5-ml. portion of a sterile aqueous solution, and the treated flesh stored at 0.5 or 10°C. Direct bacterial counts (Tarr 1943) and determinations of the amount of $NaNO_2$ present were made at stated intervals. The results of three different experiments using lingcod (*Ophiodon elongatus*) and spring salmon (*Oncorhynchus tschawytscha*) are recorded in tables VI, VII and VIII.

It will be seen that while $NaNO_2$ inhibited growth in all cases, the effect of $NaNO_3$ was either negative, or feeble and inconsistent. Thus the results given

Table VI. Effect of $NaNO_3$ and $NaNO_2$ on rate of bacterial increase in lingcod flesh stored at 0.5°C.

Treatment	Bacteria (no. per g. of flesh) after			$NaNO_2$ (p.p.m.) after		
	4 days	8 days	11 days	4 days	8 days	11 days
Control	420,000	420×10^6	$1,400 \times 10^6$	0	0	0
0.02% $NaNO_2$	130,000	3.8×10^6	$2,500 \times 10^6$	206	206	216
0.02% $NaNO_2$ + 0.1% $NaNO_3$	190,000	2.5×10^6	$1,500 \times 10^6$	214	212	208
0.02% $NaNO_2$ + 0.5% $NaNO_3$	340,000	4.2×10^6	$1,100 \times 10^6$	202	206	200
0.02% $NaNO_2$ + 1.0% $NaNO_3$	210,000	2.3×10^6	440×10^6	220	212	204

TABLE VII. Effect of $NaNO_3$ and $NaNO_2$ on rate of bacterial increase in spring salmon flesh stored at 0.5°C.

Treatment	Bacteria (no. per g.) after		$NaNO_2$ (p.p.m.) after	
	8 days	15 days	8 days	15 days
Control	74×10^6	$15{,}000 \times 10^6$	0	0
0.02% $NaNO_2$	270,000	30×10^6	198	205
0.02% $NaNO_2$ + 0.1% $NaNO_3$	530,000	30×10^6	190	195
0.02% $NaNO_2$ + 0.5% $NaNO_3$	110,000	10×10^6	198	198
0.02% $NaNO_2$ + 1.0% $NaNO_3$	420,000	16×10^6	220	210

TABLE VIII. Effect of $NaNO_3$, $NaNO_2$ and NaCl on rate of bacterial increase in lingcod flesh stored at 10°C. for 3 days.

Treatment	Direct counts ($\times 10^6$ per ml.)	$NaNO_2$ (p.p.m.)
Control	6,400	0
0.02% $NaNO_2$	200	188
0.02% $NaNO_2$ + 1% $NaNO_3$	280	448
0.02% $NaNO_2$ + 1% NaCl	280	168

in table VI indicated that 1 per cent $NaNO_3$ slightly improved the bacteriostatic effect of $NaNO_2$, while those given in table VII showed a very slight neutralization of the inhibitory effect of $NaNO_2$, but that this was just as marked with 1 per cent NaCl as with $NaNO_3$. At 0.5°C., $NaNO_3$ was not appreciably reduced to $NaNO_2$ in either of two experiments, though in the one in which fish flesh was stored at 10°C. the $NaNO_3$ was slightly reduced. The results obtained indicated that nitrate has no important bacteriostatic properties as regards fish spoilage bacteria, and that it is but slowly reduced even in stale fish flesh.

SUMMARY

The amount of $NaNO_2$ formed by bacterial reduction of $NaNO_3$ in broth medium depended on the pH of the medium. When the pH was kept near 6 either by the acid formed from added glucose, or by addition of sterile HCl, nitrite formation was strongly suppressed, and the medium soon contained no viable bacteria. On the other hand, when the pH was permitted to rise unchecked the medium became quite alkaline, and, with the two cultures studied, 1,800 to 6,950 p.p.m. of $NaNO_2$ was formed from 10,000 p.p.m. of $NaNO_3$ under different conditions, the cultures remaining viable for over 3 months. Toluene immediately stopped bacterial increase, and shortly after its addition no increase in $NaNO_2$ was noticed.

The growth of both nitrate-reducing and non-nitrate-reducing fish spoilage

bacteria was retarded by 0.02 per cent $NaNO_2$ in acid medium; but $NaNO_3$ in from 0.1 to 1.0 per cent concentration had either no effect, or a relatively trivial and variable effect on their growth either in the presence or absence of $NaNO_2$.

In from 0.1 to 1.0 per cent concentration, $NaNO_3$ had either no effect, or a relatively trivial and variable effect on the bacteriostatic action of 0.02 per cent $NaNO_2$ in fish muscle. In the experiments reported $NaNO_3$ was either not, or only slightly reduced to nitrite in spoiling fish muscle.

ACKNOWLEDGMENT

I wish to thank Miss F. M. Lew for her technical assistance in the course of this work.

REFERENCES

HOLM, G. E., AND J. M. SHERMAN. *J. Bact.*, **6**, 511-519, 1921.
JENSEN, L. B. Microbiology of meats. Garrard Press, Champaign, Ill., 1-152, 1942.
LINDSEY, G. A., AND C. M. RHINES. *J. Bact.*, **24**, 489-492, 1932.
STICKLAND, L. H. *Biochem. J.*, **25**, 1543-1554, 1931.
TANNER, F. W., AND F. L. EVANS. *Zbl. Bakt.*, Abt. II, **89**, 48-54, 1933.
TARR, H. L. A. *J. Fish. Res. Bd. Can.*, **4**, 367-377, 1940.
J. Fish. Res. Bd. Can., **5**, 265-275, 1941.
J. Fish. Res. Bd. Can., **6**, 74-89, 1942.
J. Fish. Res. Bd. Can., **6**, 119-128, 1943.
QUASTEL, J. H., M. STEPHENSON AND M. D. WHETHAM. *Biochem. J.*, **19**, 304-317, 1925.
WHITE, W. H. *Can. J. Res.*, **D17**, 125-136, 1939.
WOODS, D. D. *Biochem. J.*, **32**, 2000-2012, 1938.

Reduction of Trimethylamine Oxide by Bacteria
2. The Micrococcaceæ

By Elizabeth A. Baird and A. J. Wood
Atlantic Fisheries Experimental Station

(*Received for publication January 3, 1944*)

ABSTRACT

None of the recognized Micrococcaceae examined reduce trimethylamine oxide, although unidentified Micrococci from fish do so.

The present paper is a part of a broader study of the action of bacteria on trimethylamine oxide. The general methods and culture medium used in the present work were identical with those described in the first paper of this series, which dealt with trimethylamine formation by the Enterobacteriaceae (Wood and Baird 1943).

SELECTION OF CULTURES

Identified Micrococci and Staphylococci were obtained from the American Type Culture collection through the courtesy of Dr. C. P. Hegarty. Strains of species from the four genera of this family were obtained from a number of laboratories. These donations are acknowledged with thanks. The Micrococci from fish were isolated in this laboratory.

I. Genus *Micrococcus*

The following species and numbers of strains for the various species have been studied.

Micrococcus catarrhalis	2	*Micrococcus candidus*	1
" *butyricum*	1	" *citreus*	1
" *roseus*	3	" *flavoroseus*	1
" *cinnebareus*	1	" *lysodeikticus*	1
" *freudenreichii*	2	" *piltonensis*	1
" *luteus*	3	" *rubens*	1
" *flavus*	2	" *sphaeroides*	1
" *aurantiacus*	1	" *cereus*	1
" *varians*	1	*Rhodococcus agilis*	1
" *conglomeratus*	1	" *rhodocrous*	2
" *perflavus*	1	" *carolinus*	1
Micrococcus ureae	1	" *roseus*	12
" *candicans*	3		

J. Fish. Res. Bd. Can. 6 (3) 1944.
Printed in Canada

The nomenclature used above is that given by the donors of the cultures. All of the above cultures proved to be unable to produce trimethylamine from its oxide. Trimethylamine oxide reducing Micrococci are known (Tarr 1939, Sigurdsson and Wood 1942) to exist. The positive cultures isolated from fish muscle have thus far not yielded to classification on the basis of present systems of nomenclature.

II. Genus *Staphylococcus*

The following numbers of strains of Staphylococci of the species indicated have been tested and found to be unable to reduce trimethylamine oxide: *Staphylococcus aureus* 37; *S. albus* 9; *S. citreus* 5.

III and IV. Genera *Gaffkya* and *Sarcina*.

Six strains of *Sarcina lutea* and one strain of *Gaffkya tetragena* were tested and found to be unable to produce trimethylamine.

SUMMARY

Many of the bacteria included in the family Micrococcaceae have been tested for their ability to reduce trimethylamine oxide to trimethylamine. In direct converse to the Enterobacteriaceae (Wood and Baird 1943) most of the Micrococcaceae appear to be unable to produce trimethylamine. Future work may reveal species that are exceptions to this general finding, since trimethylamine-positive Micrococci have been isolated from fresh and salted fish.

REFERENCES

Sigurdsson, G. J., and A. J. Wood. *J. Fish. Res. Bd. Can.*, **6**, 45-52, 1942.
Tarr, H. L. A. *J. Fish. Res. Bd. Can.*, **4**, 367-377, 1939.
Wood, A. J., and E. A. Baird. *J. Fish. Res. Bd. Can.*, **6**, 194-201, 1943.

Racial Characteristics and Migratory Habits in *Salmo gairdneri*

By Ferris Neave
Pacific Biological Station

(*Received for publication July 9, 1943*)

ABSTRACT

A significant difference exists between the average scale counts of anadromous and non-sea-going *Salmo gairdneri* of the Cowichan river system, B.C. Differences in scale number and in migratory habit are maintained in hatchery-raised offspring of the two types and are considered to be founded upon inherited characteristics.

The steelhead and rainbow trout of the coastal region of British Columbia are at the present time commonly regarded as constituting a single species, *Salmo gairdneri* Richardson. As is well known, certain individuals in the course of their life history migrate to and from the sea, while others remain permanently in fresh water.

Both these habits are found among the fish inhabiting the Cowichan river system, Vancouver island. In these waters the anadromous, or sea-going, type provides fishing at two stages of its life-history:—(1) the younger fish enter into the "trout" catch, especially during the period of their migration to salt water, and (2) older individuals provide "steelhead" fishing on their return from the sea. This anadromous type of fish occurs throughout the length of the Cowichan river, up to its source in Cowichan lake, and in certain tributaries of the latter. The "resident" fish are found mainly in the upper portion of the river and in the lake, where they constitute a large proportion of the trout taken by anglers.

The question as to whether the sea-going and resident habits are the result of mere individual variation, of differences in environment or of racial characteristics is of evident scientific interest and is, moreover, directly related to certain problems of propagation and conservation, both in these waters and elsewhere.

For convenience, the terms "steelhead" and "rainbow trout" are used in the present account to designate the anadromous and non-sea-going individuals respectively.

LIFE-HISTORY

The nature of the evidence may be better understood in the light of a brief statement regarding the life-history of the species in the upper part of the river system, where fish of both types are plentiful.

Young trout are numerous at all seasons in the upper reaches of the river. Recaptures of marked and tagged individuals have shown irregular local movements in and out of small tributaries and between lake and river. A definite

J. Fish. Res. Bd. Can. 6 (3) 1944.
Printed in Canada.

migration of smolts down the river takes place in the spring, consisting of both one-year-old and two-year-old fish. At about the same time (April to June) other individuals, mainly two years of age and older, migrate upstream into the lake, where they spend the summer. In September or October trout from the lake reappear in the river, where they are to be found throughout the winter. Those which are mature spawn there, chiefly from January to March, often on the same grounds and at the same time as adult steelheads which have come up from the sea during the late autumn and winter.

SEPARATION OF TYPES

Scale Counts

Since 36 smolts captured at various times near the river mouth were all in their third spring (i.e. a little more than two years old) or younger, and since an examination of the scales of 555 adult steelheads indicated that less than one per cent had remained in fresh water for a longer period, it was assumed that fish which had definitely exceeded this age without going to sea could, as a group, be regarded as permanent dwellers in fresh water. A comparison is presented in the first half of table I between scale counts made on a series of such fish (approximately three to six years old), here called "rainbows", and on a series of steelheads of similar ages, but larger, taken from the same waters, and recognized as sea-run from examination of the scales as well as from size. Counts were made both on the lateral line and at a level about a third of the distance between the lateral line and the dorsum.

The difference of the means in both cases is highly significant, being about nine and eleven times the standard error respectively.

In order to obtain evidence as to whether these differences were due to environmental conditions, for example, temperature during early development, collections of steelhead eggs and of rainbow eggs were taken at the same time and place in February, 1940, and reared in the same water supply at the Cowichan lake hatchery until the following September, when counts were made on samples from each lot. The results are given in the second half of table I. In this instance again, the rainbow trout showed a significantly lower average scale count, the differences of the means being about six times and four and one-quarter times the standard errors.

It is reasonable to conclude from these data that the two types of fish represent different populations and that hereditary factors affect the number of scales.

Marking Experiments

Further evidence regarding migratory tendencies has been sought through the liberation of marked fingerlings of known parentage.

In 1938 and 1939 eggs were collected only from steelhead parents. These eggs were taken during January, February and March and the young fish were released, in lake and river, eight to twelve months later, after removal of the adipose and right ventral fins. Recoveries were as follows:

Brood year	Number liberated	Recoveries	
		Juvenile	Sea-run
1938	7,613	12	2
1939	35,445	28	9

Of the 40 fish which had not been to sea (juveniles), 38 were caught before the end of their third spring, i.e. within the period commonly spent in fresh water

TABLE I. Frequency distribution of scale counts made on Cowichan river rainbow trout and steelheads. d = standard deviation. d_M = standard error.

No. of scales	"Wild" fish				Hatchery fish			
	Lateral line		Above lateral line		Lateral line		Above lateral line	
	Rainbow	Steelhead	Rainbow	Steelhead	Rainbow	Steelhead	Rainbow	Steelhead
114	2	–	–	–	–	–	–	–
115	1	–	1	–	–	–	–	–
116	2	–	1	–	–	–	–	–
117	3	–	3	–	–	–	–	–
118	7	–	1	–	–	–	–	–
119	10	3	9	–	–	–	1	–
120	15	2	3	–	4	–	–	–
121	10	4	10	–	6	1	1	1
122	5	8	4	–	5	–	5	–
123	3	10	5	2	5	3	1	–
124	3	14	10	–	4	4	2	1
125	–	3	5	1	1	3	4	1
126	–	5	2	4	–	4	3	–
127	–	2	2	5	–	1	–	2
128	–	–	2	2	–	2	4	2
129	–	1	1	5	–	3	–	2
130	–	–	2	2	–	1	1	1
131	–	–	–	4	–	–	1	4
132	–	–	–	3	–	–	1	1
133	–	–	–	3	–	–	1	3
134	–	–	–	6	–	–	–	–
135	–	–	–	1	–	–	–	1
136	–	–	–	5	–	–	–	1
137	–	–	–	1	–	–	–	2
138	–	–	–	4	–	–	–	–
139	–	–	–	2	–	–	–	–
140	–	–	–	–	–	–	–	–
141	–	–	–	1	–	–	–	–
——								
159	–	–	–	1	–	–	–	–
No. of fish	61	52	61	52	25	22	25	22
Mean..	119.72	123.29	122.3	132.23	122.08	125.65	125.48	130.4
d.....	2.196	2.077	3.23	5.81	0.415	2.343	3.52	4.06
d_M....	0.284	0.291	0.417	0.812	0.085	0.512	0.72	0.886

by sea-going fish. The other 2 were taken on July 4 and August 3 of their third year, these dates being one to two months later than the close of the usual migratory period. One fish was recaptured twice, once on March 26, 1942, close to the point of liberation at the upper end of the river, and again one month later in brackish water at the river mouth.

The number of sea-run fish recovered cannot be regarded as an indication of the percentage which actually returned, as time and facilities for the capture of large steelheads were very limited. Indications are that marked sea-run steelheads of the 1939 brood year may have been quite numerous in 1943, since 9 were obtained in a total of only 19 fish caught by gill-net in the neighbourhood of the hatchery.

It is evident that these fish of steelhead parentage disappeared at an early age from the localities at which they were liberated and that at least some of them certainly went to sea.

In 1940 both rainbow and steelhead eggs were obtained from fish netted in the river. The resulting fingerlings were released in January, 1941. Details of these plantings and the number of subsequent recoveries are given in the following table.

Type of parent	Rainbow	Steelhead	Steelhead
Mark	Both ventrals	Dorsal and left ventral	Adipose and right ventral
Number released	2,976	2,976	34,800
Place released	River	River	Lake
Recoveries—			
In lake	8	0	1
In river and its tributaries	49	8	11
Total	57	8	12
Approximate no. of months after deposition of eggs	14-37	11½-26½	15-28

Particular interest attaches to the experiment involving the planting of equal numbers of fish from each type of parent. These were liberated at the same time and place. Both lots appeared quite healthy at the time of liberation, the mortality during the previous month having been less than 0.1% in both cases. The length range of the rainbow offspring was from 3 to 5 inches (7.6 to 12.7 cm.), the steelheads showing a slightly greater range, from 2½ to 5 inches (6.4 to 12.7 cm.). Of the very small number of steelheads recovered from this lot, none was taken after April 17, 1942. Recoveries of rainbows, on the other hand, have continued until March 1943, 28 having been taken subsequent to the last steelhead record. Moreover, 8 of these (recovered May to September 1942) had gone upstream into the lake, their presence there at that season being in accordance with the known habit of the non-sea-going type of fish (see p. 246). The recovery in the lake of these fish, survivors from a planting of less than 3,000 fingerlings in the river, may be contrasted with the results obtained from the 34,800 steelheads

placed directly in the lake. None of the latter has been recaptured since June 6, 1942, and all but one of the 12 fish recovered had already moved down into the river.

Introduction of Kamloops Trout

While it is not proposed at the present time to enter into a discussion regarding the origin of the steelhead and rainbow types of this river system, it seems desirable to state that a non-indigenous member of the *Salmo gairdneri* series, the Kamloops trout of the interior of British Columbia, has been planted in these waters at various times between 1922 and 1934, and again between 1938 and 1942. The question naturally arises as to what influence, if any, these introductions have had on the existing populations of steelhead and rainbow trout.

The writer believes that neither of the two types discussed in the present paper owes its origin to, or has been markedly influenced by, the Kamloops trout. The following considerations may be cited:

(1) Both sea-run and resident fish were well known in the Cowichan before any Kamloops trout were introduced.

(2) All Kamloops trout introduced during the recent period have been marked distinctively and did not enter into the samples taken. Recoveries to date indicate a poor survival and no fish of breeding age have been found. A planting of 33,000 Kamloops trout of brood year 1939 was made in the lake and river at a considerably larger average size than the similar number of steelheads. These yielded only 20 returns, all within a period of one year from the time of liberation. While this figure compares fairly well with the recoveries of juvenile steelheads of the same year class, there has been no indication that the Kamloops trout avoided capture by going to sea. Recoveries from other plantings of Kamloops trout have been negligible.

(3) Kamloops trout reared from a late egg stage in the Cowichan lake hatchery show a much higher average scale count than even local steelheads (Neave 1943) and are still further removed in this respect from local rainbows, with which they might have been supposed to have a greater affinity.

DISCUSSION

Status of Types

It is concluded that the types of *Salmo gairdneri* found in the upper part of the Cowichan river system represent, in the main, two separate indigenous races, the tendency to go to sea or to remain in fresh water being largely controlled by hereditary factors. In this connection it may be pointed out that Landgrebe (1941) has recently suggested that the anadromous and non-sea-going types of brown trout (*S. trutta* L.) are genetically distinct, the difference between them being "associated with functional activity of the thyroid gland".

It is not contended that the offspring of sea-run fish never remain in fresh water until maturity. If they do, the situation would be analogous in some respects to that provided by the sockeye in Cultus lake (Ricker 1938, 1940), in which "residuals" from sea-run fish occur in addition to a self-maintaining stock

of fresh-water kokanees. The present evidence from the Cowichan suggests that residual steelheads, if they occur, are very scarce.

Problems of Conservation and Propagation

From the standpoint of conservation it is evident that two self-perpetuating stocks of fish must be treated as if they were different species. If the conclusions reached in the present instance are correct, measures taken to protect or increase one type will not necessarily benefit the other. For example, the eight-inch (20.3 cm.) size limit at present in force ensures the escape of a large percentage of steelheads to the sea, but the maintenance of the steelhead runs will not protect the rainbow trout in a corresponding manner and might, indeed, be detrimental to the latter, if the steelheads monopolized the spawning and nursery grounds. The rainbow undoubtedly has to withstand a relatively heavier fishing drain than the steelhead in these waters.

The possible value of the Cowichan river rainbow trout for introduction elsewhere can be mentioned briefly. The extensive migratory tendencies which have nullified the introduction of "rainbow trout" in many localities have evoked a frequent desire for a "non-migratory strain". The U.S. Bureau of Fisheries in 1936 and 1937 made two expeditions to a remote part of Mexico in order to obtain a strain of rainbow trout which, because of physical conditions, were unable to migrate for any great distance (Needham 1938). The Cowichan rainbow trout makes a very short migration and the evidence indicates that, in its native locality, this characteristic is hereditary. The present writer will not undertake to predict the result of introducing trout into other waters. It may be suggested, however, that a fish which does not go to sea even when it has the opportunity deserves as much consideration as one which has been prevented from so doing by physical barriers. Additional recommendations might be found in the facts that the Cowichan river rainbow exists naturally in relatively warm water and in the face of competition from several other species of salmonids.

SUMMARY

A significant difference has been found between the average scale counts of anadromous and non-sea-going *Salmo gairdneri* of the Cowichan river system. This difference was found to persist in fish raised under the same environmental conditions.

Experiments with marked fish have shown that the offspring of anadromous parents disappeared from the fresh-water localities in which they were planted before or during their third spring. Some of these fish subsequently reappeared in these localities as adult sea-run steelheads.

Marked offspring of non-sea-run parents have remained in fresh water until their fourth spring. At least some of these fish migrated upstream to Cowichan lake during or prior to their third summer, in contrast to migrating seaward.

It is concluded that in this river there are two distinct races of *S. gairdneri*, the migratory habits of which are largely dependent on inherited characteristics.

REFERENCES

LANDGREBE, F. W. The role of the pituitary and the thyroid in the development of teleosts. *J. Exper. Biol.*, **18**, 162-169, 1941.

NEAVE, F. Scale pattern and scale counting methods in relation to certain trout and other salmonids. *Trans. Roy. Soc. Can.*, **37** (5) 79-91, 1943.

NEEDHAM, P. R. Notes on the introduction of *Salmo nelsoni* Evermann into California from Mexico. *Trans. Amer. Fish. Soc.*, **67**, 139-145, 1938.

RICKER, W. E. "Residual" and kokanee salmon in Cultus lake. *J. Fish. Res. Bd. Can.*, **4**, 192-218, 1938.

On the origin of kokanee, a fresh-water type of sockeye salmon. *Trans. Roy. Soc. Can.*, **34** (5), 121-135, 1940.

Effects of Dropping on the Subsequent Hatching of Teleostean Ova

By Helen I. Battle
Department of Zoology and Applied Biology
University of Western Ontario

(*Received for publication September 16, 1943*)

ABSTRACT

Two demersal eggs (*Gasterosteus aculeatus*, *Fundulus heteroclitus*) and one pelagic type (*Enchelyopus cimbrius*), subjected to uniform mechanical shock by dropping, evidenced a susceptibility during cleavage and blastula formation which decreased as gastrulation proceeded and became markedly reduced as epiboly terminated. Eggs of *Gasterosteus* show greatest sensitivity, those of *Fundulus* least. Abnormality of the body axis appears to result from shock.

The present paper is a record of experiments designed to determine the susceptibility of various types of fish eggs to external influences of a purely physical nature during various phases of the incubation period. The eggs of three teleostean fishes are available simultaneously at St. Andrews, New Brunswick, during the summer months. Two of these are demersal, namely those of the three-spined stickleback, *Gasterosteus aculeatus* (L), and the mummichog or killifish, *Fundulus heteroclitus* (L.), while those of the four-bearded rockling, *Enchelyopus cimbrius* (L.) are pelagic. The stickleback offers a classic example among fishes of parental care of eggs and young, a fact which leads to the suggestion that agitation of the eggs, or shock produced by some mechanical means may have a deleterious effect upon development. On the other hand the eggs of *Fundulus* are particularly hardy in type, and protected by an exceptionally heavy chorion bearing numerous adhesive elastic threads. The rockling egg has a thin chorion similar to that of the stickleback except that it is non-adhesive so that the eggs float singly in the water instead of clinging in clumps as in the case of the other species.

METHOD

The demersal eggs were obtained by stripping adults. Mature stickleback were seined from tidal pools near the laboratory as required, while killifish were seined at Birch cove and kept in laboratory aquaria of running water. The pelagic rockling ova were taken in plankton tows at station 848 in the middle of Passamaquoddy bay, hence stages earlier than the many-celled (64+) blastoderm were not available for experimental work. All the eggs develop normally at the temperature of the laboratory (15° to 19°C.) and were subject to routine dropping as soon as any desired stage was attained.

J. Fish. Res. Bd. Can. **6** (3) 1944.
Printed in Canada

The relative sensitivity of the three types of eggs to mechanical shock was determined by causing them to drop from different heights in a manner similar to that used by Rollefsen (1932) for cod eggs. The eggs were introduced into a pipette with a discharge which permitted approximately five eggs to fall at each drop onto a piece of bolting silk tightly stretched across a metal frame and resting at the base of a glass cylinder 65 cm. long and 6 cm. in diameter. The distance of the fall was regulated by adjusting the pipette to distances of 20, 40 and 60 cm. from the bolting silk. After the fall the eggs were transferred (by turning the bolting silk frame upside down) into four-ounce bottles containing seawater and development was allowed to proceed at laboratory temperature. Twenty eggs were used in each experiment and a control of an equal number was run for each species with every fresh lot of eggs. In each instance record was made both of the percentage hatched, and also of the percentage of the larvae normal in external appearance, especially as to body axis. The control given for each species is an average calculated from three to five sets of eggs used throughout the course of the experiment.

RESULTS AND DISCUSSION

Although the numbers of eggs available for each experiment were relatively small, the results (fig. 1) indicate primarily that the eggs of the three species are not equally susceptible to mechanical shock. The eggs of *Gasterosteus* throughout early stages up to the completion of epiboly are more susceptible to shock than are those of the other two forms, judging from the smaller percentage of larvae hatching and the relatively low percentage of those hatched which are normal. Both *Gasterosteus* and *Enchelyopus* have a very thin chorion, which readily allows for pressure on the egg contents. In early stages excess pressure ruptures the thin cytoplasmic layer enclosing the yolk sphere, permitting the yolk to flow out into the perivitelline space, and simultaneously the embryonic area may disintegrate. If the yolk has been overgrown by embryonic tissue, the sphere is less easily ruptured, but the embryonic axis may show considerable contortion. In *Fundulus* the extremely heavy character of the chorion is a factor in preventing shock due to impact and hence these eggs throughout development show less susceptibility than the other two forms. The eggs of *Enchelyopus* occupy an intermediate position between the other two forms, since, although the chorion is very thin, development may proceed after dropping, with, however, a low percentage of normality, especially during the many-celled blastoderm and earlier epibolic stages. It has been shown previously (Battle 1929) that these are critical stages in development since subjections to extremes of temperature or salinity result in an abnormal pattern of development.

It may also be observed that the eggs in any one form are not equally susceptible to mechanical shock at all stages of development. Those of *Gasterosteus* may be dropped prior to fertilization without interfering entirely with the latter process although the percentage of normal larvae produced is small. The eggs become much more sensitive immediately after fertilization, and reach a high level of sensitivity during cleavage and blastula formation. As the yolk sphere is covered by embryonic tissue during gastrulation, sensitivity decreases and

becomes relatively low on the completion of epiboly as the embryonic axis is definitely delineated.

The eggs of *Fundulus* may be dropped prior to fertilization with relatively low loss and with production of few abnormal larvae. Sensitivity increases after fertilization, apparently reaching a high level also during the many-celled blastodermic stage, but decreases progressively as epiboly proceeds. At no stage during early development is the sensitivity as great as in the stickleback egg.

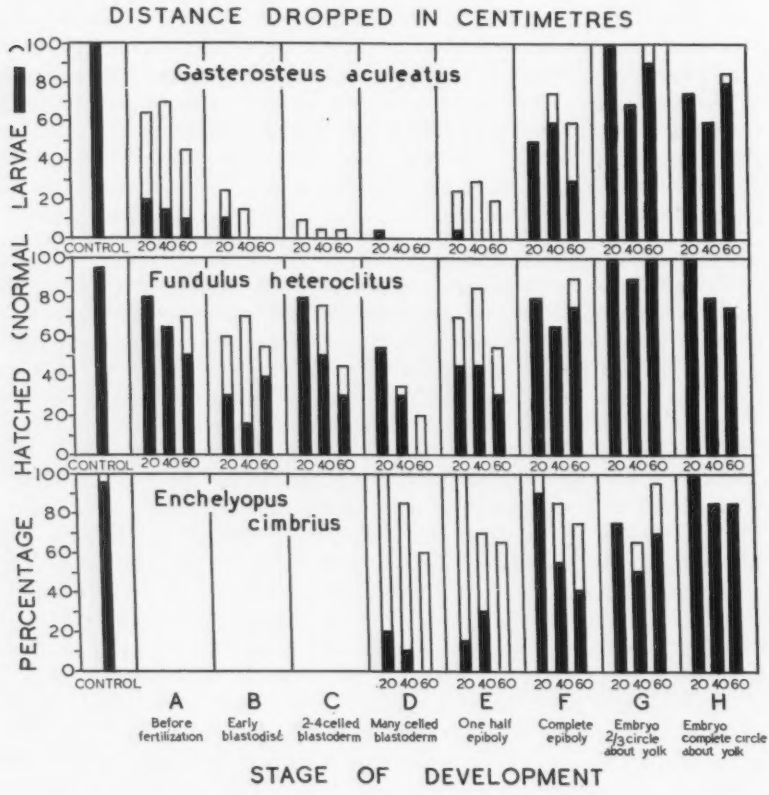


FIGURE 1. Effects of falling through distances of 20, 40 and 60 cm. on subsequent hatching of ova of *Gasterosteus aculeatus*, *Fundulus heteroclitus* and *Enchelyopus cimbrius*, subjected during typical developmental periods. Outline rectangles represent the percentage hatched and solid rectangles the percentages of those hatched which are normal.

Unfortunately no early stages of the eggs of *Enchelyopus* were available. Since, however, the effect of agitating and dropping the eggs of the cod, a closely allied member of the Gadidae, has previously been reported in some detail by Rollefsen (1930, 1932) it may be assumed that the rockling would respond in a similar manner. Using eggs taken by net or stripped from the adult, he found

that susceptibility increases throughout segmentation but decreases progressively as the blastoderm covers the yolk in the process of epiboly. Sensitivity of the eggs of *Enchelyopus* is greatest during the many-celled blastoderm, slightly less when the yolk is partially overgrown by the embryonic tissue and progressively less as the yolk is enclosed and the embryonic axis lengthens.

During the process of epiboly, as the yolk is overgrown by the embryonic tissue, there comes to be a progressive decrease in sensitivity in all three species. In addition the percentage of normally developed larvae of *Enchelyopus* increases and now approximates that occurring in the demersal ova, whereas in earlier stages it was extremely low as compared to that of *Fundulus*. Following the completion of epiboly when the embryonic axis commences to encircle the margin of the yolk, the sensitivity of all the ova to mechanical shock decreases since the yolk sphere is no longer readily ruptured. This is particularly true for both these demersal eggs where the yolk sphere becomes completely covered by a heavy vascular plexus.

A number of investigators besides Rollefsen (1930, 1932) have observed that the susceptibility of other fish eggs to external influence of a purely physical nature changes during the incubation period. Hein (1907) subjected eggs of the trout (*Salmo fario*) to various types of adverse conditions such as pressure and shock, at different intervals after fertilization. He observed a gradual decrease in susceptibility up to the closure of the blastopore (13 to 17 days) and a subsequent increase just prior to or at hatching. Scheminzky (1922) and Scheminzky and Gauster (1924) obtained similar results when eggs of the trout *Salmo lacustris* were exposed to electric currents. Kyuzo Hata (1927) tested the influence of vibrations upon the eggs of *Oncorhynchus masou* Brevoort, and found that immediately after fertilization and again at the appearance of the embryonic axis and optic vesicles, the eggs were more susceptible than in intermediate and older stages.

Possibly because of the small numbers of eggs used in each experiment, in only certain instances is it possible to observe a direct relationship between the percentage hatching and the normality of the larvae and the severity of the impact, i.e. upon the distance through which the eggs were dropped. Reference to figure 1 does indicate, however, such a relationship between the height of fall and the number of normally hatched larvae when the following stages were subjected: *Gasterosteus*,—early blastodisc, two four-celled blastoderm, and many-celled blastoderm; *Fundulus*,—two-four celled blastoderm, many-celled blastoderm, and embryo complete circle about yolk; *Enchelyopus*,—many-celled blastoderm, complete epiboly, and embryo complete circle about yolk.

Abnormalities of the larvae hatched following dropping of the various developmental stages appeared to be confined to the embryonic axis. This involved contortion of the notochord, resulting in various anomalies from a simple humpback condition or a mere twisting of the postanal region of the body to a complex cork-screwing of the trunk and tail. The results would seem to suggest that it may be well worth while to investigate further effects of mechanical shock on embryonic processes.

REFERENCES

BATTLE, HELEN I. Effects of extreme temperatures and salinities on the development of *Enchelyopus cimbrius* (L.). *Contr. Canad. Biol. Fish.*, N.S. **5**, 109-192, 1929.

HATA, KYUZO. On the influence of four kinds of vibration upon the eggs of *Oncorhynchus masou* Brevoort. *J. Imper. Fish. Inst. Tokyo*, **23**, 74-78, 1927.

HEIN, WALTER. II. Zur Biologie der Forellenbrut. (2) Über die absolute Druckfestigkeit der Bachforelleneier. (3) Über die Wirkungen von Druck, Stoss und Fall auf die Entwicklung der Bachforelleneier. *Allg. Fischereizeitg* **32**(18), 383-387, 1907.

ROLLEFSEN, GUNNAR. V. Observation on cod eggs. *Rapp. Cons. Explor. Mer.* **65**, 31-34, 1930. The susceptibility of cod eggs to external influences. *J. Conseil* **7**(3), 367-373, 1932.

SCHEMINZKY, FERD. Über die verschiedene Empfindlichkeit der Forelleneier während ihrer Entwicklung dem elektrischen Strom gegenüber. *Biochem. Zs.* **132**, 154-164, 1922.

SCHEMINZKY, FERD., UND FRITZI GAUSTER. Beiträge zur physikalisch-chemischen Biologie der Forellenentwicklung. 1. Mitteilung: Die Schädigung der Membran des Forelleneies durch den elektrischen Strom. *Arch. mikr. Anat. Entw.* **101**, 1-39, 1924.

Chemical Inhibition of Growth of Fish Spoilage Bacteria

By H. L. A. Tarr
Pacific Fisheries Experimental Station

(*Received for publication October 26, 1943*)

ABSTRACT

Inhibition of the growth of fish spoilage bacteria in naturally contaminated fish muscle, and in some instances of pure cultures of such organisms cultivated on laboratory media, by penicillic acid, 4-methoxy-2 : 5 toluquinone, methyl formate, ethyl formate, ethylene oxide, propylene oxide, methyl ether, ethyl ether, chloroform, ethylene dichloride, ethyl chloride, 1 : 4 dioxane, Chloramine B, Chloramine T, a mixture of isomeric glycerol formals, sodium chlorite, sodium benzoate, sodium nitrite, and one patent fish preservative was investigated. The results are discussed and are summarized in detail.

The value of a number of different chemical compounds in inhibiting growth of fish spoilage bacteria in culture media and in fish flesh itself was described in previous publications (Tarr and Sunderland 1939, 1940; Tarr 1941, 1942). The bactericidal or bacteriostatic action of various chemical compounds, many of which were not previously investigated, is recorded herewith.

EXPERIMENTS WITH ISOLATED BACTERIAL CULTURES

The cultures used were those employed in previous experiments (Tarr 1939).

Penicillic acid (5-methylene 4-keto 3-methoxy 2-hexenoic acid) was prepared by the action of *Penicillium cyclopium* Westling on glucose as described by Birkinshaw, Oxford and Raistrick (1936). The ***4-methoxy-2:5 toluquinone*** was prepared by Ashley's method (1937). Solutions of these compounds of the required strength were made by dissolving them in boiling sterile distilled water (Oxford, Raistrick and Smith 1942). Sterile 16 × 150-mm. tubes received 2 ml. of Bacto nutrient broth (double the normal strength), 0.5 ml. of 0.2 M phosphate buffer (different pH values), 1 ml. of an aqueous solution of penicillic acid or 4-methoxy-2 : 5 toluquinone of the desired strength (water in controls) and 0.5 ml. of a saline suspension of cells of the organism being studied. The saline suspensions of the test organisms were prepared by transferring a portion of a 1- or 2-day-old culture of the organism in Bacto nutrient broth into sterile 0.1 per cent sodium chloride, the number of bacteria present being determined directly using a Petroff-Hausser counting chamber. The inoculated tubes were incubated at 25°C., the amount of visible growth being recorded at stated intervals.

The results of experiments using one species of *Micrococcus* and two of *Achromobacter* (tables I and II) showed that the bactericidal or bacteriostatic

J. Fish. Res. Bd. Can. **6** (3) 1944.
Printed in Canada

action of both compounds varied with the cultures used, and in all instances was more pronounced in acid than in neutral or faintly alkaline medium. With the exception of culture 1 (*Micrococcus* sp.) neither compound exerted a very pronounced inhibition in 0.001 per cent concentration, 0.01 per cent being, however, usually very active in this respect. On the whole 4-methoxy-2 : 5 toluquinone exhibited similar, though more marked, inhibitory action than did penicillic acid.

Methyl bromide has marked insecticidal properties (Dudley and Neal 1942), and inhibits the growth of yeasts which contaminate dates (Mrak 1941), but as

TABLE I. Effect of penicillic acid on the growth of culture 22 (*Achromobacter* sp.), culture 16 (*Achromobacter* sp.), and culture 1 (*Micrococcus* sp.). Inocula 8.9×10^6, 0.7×10^6, and 2.3×10^6 organisms (or clumps of cells for culture 1) respectively.

Culture no.	Initial pH of medium	Controls			0.01% penicillic acid			0.001% penicillic acid		
		1 day	2 days	2 weeks	1 day	2 days	2 weeks	1 day	2 days	2 weeks
22	5.90	+++	+++	+++	–	–	–	++	+++	+++
"	6.00	+++	+++	+++	–	–	–	++	+++	+++
"	6.20	+++	+++	+++	–	–	+++	++	+++	+++
"	6.45	+++	+++	+++	–	–	+++	++	+++	+++
"	6.75	+++	+++	+++	–	++	+++	+++	+++	+++
"	7.20	+++	+++	+++	++	+++	+++	+++	+++	+++
"	7.55	+++	+++	+++	++	+++	+++	+++	+++	+++
"	7.80	+++	+++	+++	++	+++	+++	+++	+++	+++
16	5.90	+	+++	+++	–	–	–	–	++	+++
"	6.00	+	+++	+++	–	–	+++	+	++	+++
"	6.20	+	+++	+++	–	–	+++	+	+++	+++
"	6.45	+	+++	+++	–	+	+++	++	+++	+++
"	6.75	++	+++	+++	–	+	+++	++	+++	+++
"	7.20	+++	+++	+++	+	++	+++	++	+++	+++
"	7.55	+++	+++	+++	+	+++	+++	++	+++	+++
"	7.80	++	+++	+++	+	+++	+++	++	+++	+++
1	5.90	–	–	–	–	–	–	–	–	–
"	6.00	–	–	–	–	–	–	–	–	–
"	6.20	+	++	+++	–	–	–	–	–	–
"	6.45	++	++	+++	–	–	–	–	–	–
"	6.75	++	++	+++	–	–	–	–	–	++
"	7.20	+	+	+	–	–	–	–	–	+
"	7.55	–	–	+	–	–	–	–	–	+
"	7.80	–	–	–	–	–	–	–	+	+

far as the writer is aware its bactericidal action has not been previously investigated.

Bacto nutrient agar (pH 7.1) and nutrient broth (pH 7.2) were sterilized in 3-ml. amounts in 12 × 150-mm. tubes, the agar medium being sloped prior to inoculation. Cultures 1 to 24 were grown for 24 hours at 25°C. in Bacto nutrient broth (pH 7.0) and one 2-mm. loopful of each culture was transferred to each of five broth and five agar tubes. Five complete similar sets of inoculated media were thus obtained for each of the 24 cultures, and one set at a time was treated with methyl bromide as follows. The tubes were placed in a vacuum desiccator

and subjected to reduced pressure (about 6 cm. of mercury) by means of a water pump. The desired amount of methyl bromide (measured in cm. of mercury) was then permitted to enter the desiccator, followed by air until prevailing atmospheric pressure resulted. The tubes were exposed for 30 minutes, and were then removed, incubated at 25°C., and the presence or absence of growth noted at stated intervals. The results (table III) showed that methyl bromide exerted a pronounced bactericidal action, only 12 per cent of broth and 6 per cent of agar cultures exhibiting growth after two weeks following exposure to 50 cm. of methyl

TABLE II. Effect of 4-methoxy-2 : 5 toluquinone on the growth of culture 22 (*Achromobacter* sp.), culture 16 (*Achromobacter* sp.), and culture 1 (*Micrococcus* sp.). Inocula 8.9×10^6, 0.7×10^6 and 2.3×10^6 organisms (or clumps of cells for culture 1) respectively.

Culture no.	Initial pH of medium	Controls			0.01% 4-methoxy-2 : 5 toluquinone			0.001% 4-methoxy-2 : 5 toluquinone		
		1 day	2 days	2 weeks	1 day	2 days	2 weeks	1 day	2 days	2 weeks
22	5.90	+++	+++	+++	−	−	−	++	+++	+++
"	6.00	+++	+++	+++	−	−	−	++	+++	+++
"	6.20	+++	+++	+++	−	−	−	++	+++	+++
"	6.45	+++	+++	+++	−	−	−	++	+++	+++
"	6.75	+++	+++	+++	−	−	−	++	+++	+++
"	7.20	+++	+++	+++	−	−	−	++	+++	+++
"	7.55	+++	+++	+++	−	−	−	++	+++	+++
"	7.80	+++	+++	+++	−	−	−	++	+++	+++
16	5.90	+	+++	+++	−	−	−	−	−	−
"	6.00	+	+++	+++	−	−	−	−	−	−
"	6.20	+	+++	+++	−	−	−	+	+++	+++
"	6.45	+	+++	+++	−	−	−	+	+++	+++
"	6.75	++	+++	+++	−	−	−	++	+++	+++
"	7.20	+++	+++	+++	−	−	−	++	+++	+++
"	7.55	+++	+++	+++	−	−	−	++	+++	+++
"	7.80	++	+++	+++	−	−	−	++	+++	+++
1	5.90	−	−	−	−	−	−	−	−	−
"	6.00	+	+	+	−	−	−	−	−	−
"	6.20	++	+	++	−	−	−	−	−	−
"	6.45	++	++	+++	−	−	−	−	−	−
"	6.75	+	++	+++	−	−	−	−	−	−
"	7.20	+	++	+++	−	−	−	−	−	+
"	7.55	+	+	++	−	−	−	−	−	+
"	7.80	−	−	+	−	−	−	−	−	+

bromide for 30 minutes. It was not determined whether methyl bromide would exert greater activity if applied in partial vacuum without allowing air to run in until atmospheric pressure was attained.

EXPERIMENTS WITH NATURALLY CONTAMINATED FISH MUSCLE

The following technique was used throughout for the sake of uniformity. Fresh fish were filleted carefully under clean conditions, the flesh was minced in a domestic machine, mixed thoroughly, and 95-g. portions were placed in sterile

250-ml. covered glass beakers. The various compounds studied (with the exception of those applied as gas) were dissolved to the desired concentration in sterile distilled water, 5 ml. of the resulting solution being added to, and intimately mixed with, one 95-g. portion of minced fish muscle. Special precautions had to be observed with solutions of 4-methoxy-2 : 5 toluquinone and of ethylene oxide. The former, which is sparingly soluble in cold water, was added as a hot solution, while the latter, which readily loses gas on warming, was added under cold conditions. Treated samples were stored at 0.5°C. unless otherwise stated. Direct

TABLE III. Effect of methyl bromide (MeBr) on growth

Culture no.	Genus	Culture (No MeBr)						5 cm. MeBr					
		1 day		2 days		14 days		1 day		2 days		14 days	
		A*	B*	A	B	A	B	A	B	A	B	A	B
1	*Micrococcus*	+	−	+	−	+	+	+	−	+	−	+	−
2	"	+	+	+	+	+	+	+	−	+	−	+	+
3	Yeast	+	+	+	+	+	+	+	−	+	−	+	+
4	*Micrococcus*	+	+	+	+	+	+	+	−	+	+	+	+
5	"	+	+	+	+	+	+	+	−	+	−	+	+
6	*Flavobacterium*	+	+	+	+	+	+	+	−	+	−	+	−
7	*Micrococcus*	+	+	+	+	+	+	+	+	+	+	+	+
8	"	+	−	+	−	+	+	+	−	+	−	+	−
9	*Achromobacter*	+	+	+	+	+	+	+	−	+	−	+	+
10	*Micrococcus*	+	−	+	−	+	+	+	−	+	−	+	−
11	"	+	+	+	+	+	+	+	−	+	+	+	+
12	Yeast	+	+	+	+	+	+	−	−	+	−	+	+
13	*Micrococcus*	+	−	+	−	+	+	+	−	+	−	+	+
14	Yeast	+	+	+	+	+	+	+	−	+	−	+	−
15	"	+	+	+	+	+	+	+	−	+	−	+	+
16	*Achromobacter*	+	−	+	+	+	+	+	−	+	+	+	+
17	"	+	+	+	+	+	+	+	−	+	+	+	+
18	*Micrococcus*	+	−	+	−	+	+	+	−	+	−	+	+
19	*Achromobacter*	+	+	+	+	+	+	+	−	+	−	+	+
20	*Micrococcus*	+	−	+	−	+	+	+	−	+	−	+	+
21	*Flavobacterium*	+	−	+	−	+	+	−	−	+	−	+	+
22	*Achromobacter*	+	+	+	+	+	+	+	+	+	+	+	+
23	"	+	+	+	+	+	+	+	−	+	+	+	+
24	*Micrococcus*	+	+	+	+	+	+	+	−	+	−	+	+

*Á = agar; B = broth; + = growth; − = no visible growth.

(Tarr 1943) or viable (Tarr and Bailey 1939) bacterial counts were made after a stated time.

Gas treatment of fish flesh was carried out as follows. Beakers containing the minced muscle were placed in a vacuum desiccator and subjected to a reduced pressure (3 or 6 cm. of mercury) for 2 minutes. The required amount (measured by the increase of the pressure) of the gas being studied was run in as quickly as possible, though in the case of gases which were very soluble in water (ethylene and propylene oxides) rapid solution of the gas in the muscle fluid made the

measurement of added gas only very approximate. The flesh was exposed to the gas for a given time, and then subjected to a reduced pressure (about 2 cm. of mercury) for 5 minutes in order to remove much of the gas from the fish. Air was then allowed to enter the desiccator, and the treated samples were stored at 0.5 or 10°C., direct bacterial counts being made at stated times.

The following compounds were studied in the experiments to be described: methyl formate, ethyl formate, methyl ether (purchased as 40 per cent solution in sulphuric acid) and propylene oxide (E.K. "Eastman"); ethylene glycol, pro-

of isolated bacterial cultures in broth and agar media.

10 cm. MeBr						25 cm. MeBr						50 cm. MeBr					
1 day		2 days		14 days		1 day		2 days		14 days		1 day		2 days		14 days	
A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
+	−	+	−	+	+	−	−	+	−	+	+	−	−	−	−	−	−
−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	+
−	−	+	+	+	+	−	−	+	−	+	+	−	−	−	−	−	+
−	−	+	−	+	+	−	−	+	−	+	−	−	−	−	−	+	−
−	−	+	−	+	+	−	−	−	−	+	−	−	−	−	−	−	−
+	+	+	+	+	+	−	−	+	+	+	+	−	−	−	−	+	+
−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	+	−	+	+	−	−	−	−	−	+	−	−	−	−	−	−
−	−	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−
−	−	+	−	+	+	−	−	−	−	+	+	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
−	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
−	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	+	−	+	+	−	−	−	−	−	+	−	−	−	−	−	+
−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	+	−	+	+	−	−	−	−	+	+	−	−	−	−	−	−
+	+	+	+	+	+	−	−	+	+	+	+	−	−	+	−	+	+
+	−	+	+	+	+	−	−	−	−	+	+	−	−	−	−	−	+
−	−	+	−	+	+	−	−	+	+	+	+	−	−	−	−	−	−

pylene glycol, trimethylene glycol, 1 : 4 dioxane, methyl bromide and ethylene dichloride (E.K. "Practical"); ethylene oxide, ethyl chloride and chloroform (E.K. "Technical"); Chloramine T (B.D.H.), Chloramine B (Solvay Process Co.); sodium chlorite (Mathieson Chemicals "Technical"), and sodium nitrite (B.D.H. "A.R."). A mixture of 5-m-dioxanol and 4-hydroxymethyl-1 : 3 dioxolane (isomeric glycerol formals) was kindly prepared by Dr. N. M. Carter of this Station. A sample of "Nipicide", a patent fish preservative, was kindly obtained by Mr. M. E. Stansby of the U.S. Fish and Wildlife Service. A rough analysis showed that it contained sodium benzoate and probably a chloramine.

Compounds Applied in Solution

The bacteriostatic action of most of the compounds listed above was studied in two tests, one using minced halibut muscle and 0.01 per cent of each compound, the other minced coho salmon (*Oncorhynchus kisutch*) muscle and 0.1 per cent, the treated samples being stored at 0.5°C. Direct bacterial counts were made on the samples at intervals.

The results (table IV) showed that none of the compounds studied exerted any important bacteriostatic effect in 0.01 per cent concentration. In 0.1 per

Table IV. Effect of various compounds on growth of bacteria in fish muscle.

Compound investigated	Experiment with halibut muscle using 0.1% of each compound		Experiment with coho salmon muscle using 0.1% of each compound
	Direct bacterial counts after		Direct bacterial counts after
	1 day	6 days	9 days
Controls (water)	2.5×10^6	2500×10^6	710×10^6
Ethylene glycol	2.1×10^6	2200×10^6	350×10^6
Propylene glycol	1.7×10^6	1400×10^6	1600×10^6
Trimethylene glycol	2.5×10^6	1100×10^6	1400×10^6
Glycerol formal	1.8×10^6	1200×10^6	2400×10^6
1 : 4 dioxane	3.2×10^6	2000×10^6	460×10^6
Ethylene oxide	1.8×10^6	2600×10^6	2×10^6
Propylene oxide	.....	.....	55×10^6
Ethyl formate	.....	.....	160,000
Methyl formate	.....	.....	240,000
Chloroform	1.3×10^6	1200×10^6	2200×10^6
Chloramine B	1.5×10^6	1600×10^6	680×10^6
Chloramine T	2.1×10^6	1900×10^6	1300×10^6
"Nipicide"	1.5×10^6	1500×10^6	1000×10^6
Sodium benzoate	1.6×10^6	2100×10^6	440×10^6
Sodium chlorite	2.4×10^6	950×10^6	320×10^6
Sodium nitrite	1.7×10^6	1200×10^6	28×10^6*
Ethylene dichloride	2.3×10^6	1900×10^6	.....
Penicillic acid	.....	.....	110×10^6
4-Methoxy-2 : 5 toluquinone	.....	.....	300×10^6

*0.02% sodium nitrite used in this test.

cent concentration methyl formate, ethyl formate, ethylene oxide and propylene oxide strongly retarded bacterial multiplication. Penicillic acid and 4-methoxy-2 : 5 toluquinone exerted only feeble bacteriostatic action in this concentration. Sodium nitrite (0.02 per cent) was quite effective in this respect, but none of the other compounds studied caused any important inhibition of bacterial growth. Ethylene oxide, propylene oxide, penicillic acid and 4-methoxy-2 : 5 toluquinone caused a pronounced brownish discolouration and rancid odour in the salmon flesh, while methyl and ethyl formates caused an appearance similar to that assumed by flesh treated with weak acids.

Ethylene oxide and propylene oxide have found wide industrial use as insecticides, but in recent years several patents have been granted covering their application as bactericides. Thus Gross and Dixon (1937) patented the use of ethylene oxide and propylene oxide under reduced pressure for sterilizing (or partially sterilizing) a great variety of foods ranging from fresh meats to rolled oats, as well as soils, tobacco and surgical instruments. These writers assumed that ethylene glycol was formed in moist foods during treatment. Jensen (1943 unpub.) states that ethylene oxide must not be used for processing moist foods due to danger of ethylene glycol formation. As far as the writer is aware no published information is available concerning the amount of ethylene glycol formed in moist foods following ethylene oxide treatment. Wood (unpub.) found that he could obtain fish muscle which was bacteriologically sterile by treating it with ethylene oxide gas *in vacuo*. Moreover, he discovered that such treatment did not inactivate any of a number of its normal enzyme systems which he inves-

TABLE V. Effect of ethylene and propylene oxides on the rate of bacterial increase (shown as bacteria per gram by dried count) in fish muscle.

Applied in solution—halibut muscle—storage at 0.5°C.

	Control	Ethylene oxide (%)		Propylene oxide (%)	
After 10 days	750×10^6	1 300,000	0.1 440×10^6	1 2.9×10^6	0.1 720×10^6

Applied as gases—lingcod muscle—storage at 10°C.

	Control	Ethylene oxide in cm.				Propylene oxide in cm.			
After		5	10	20	30	5	10	20	30
4 days	*$22,000\times10^6$	670×10^6	740,000	80,000	80,000	*$9,000\times10^6$	260×10^6	30×10^4	560,000
8 days		*	*	$2,700\times10^6$	720×10^6		*	*	*$6,300\times10^6$

*Putrid.

tigated. The possible value of this treatment as a method of rendering fish muscle free from bacteria for studies of its enzyme systems therefore is obvious. Ethylene oxide can be used successfully in commercial sterilization of compartively dry substances such as spices (Griffith and Hall 1938, Baer 1941) and is very much more efficient than is ethylene glycol for this purpose (Yesair and Williams 1942).

The foregoing experiment showed that ethylene and propylene oxides in 0.1 per cent concentration successfully retarded multiplication of bacteria in the flesh of fatty fish (coho salmon) but also accelerated the development of rancidity. However, ethylene oxide in this concentration did not inhibit growth of bacteria in halibut flesh, and this result was verified in the present experiment in which it was found that 0.1 per cent of ethylene or propylene oxide did not occasion any marked reduction in the rate of bacterial increase in this fish, 1 per cent being required before a pronounced decrease in the number of bacteria was observed (table V).

Compounds Applied as Gases

Minced lingcod (*Ophiodon elongatus*) was exposed to reduced pressure (3 cm. of mercury), treated for 30 minutes with different partial pressures of ***ethylene*** and ***propylene oxide*** as previously described, and stored at 10°C. Direct bacterial counts were made at intervals, the results being given in table V. It will be seen that both gases effectively retarded bacterial multiplication in the flesh, ethylene oxide being more effective than propylene oxide.

Minced red cod (*Sebastodes* sp.) flesh was exposed to reduced pressure (6 cm. of mercury) and then different samples were exposed to different concentrations of ***methyl bromide*** in the usual manner. The following viable counts were obtained on the samples immediately after treatment with the partial pressures of methyl bromide given: Control 28,000; 5 cm., 27,000; 10 cm., 24,000; 25 cm., 23,000; 50 cm., 19,000. This showed that methyl bromide effected only a very slight decrease in the number of viable bacteria in the fish muscle. Subsequent storage of the treated samples at 0.5°C. showed that this gas treatment occasioned no noticeable improvement in keeping quality as judged by the odour and appearance of the samples. A similar experiment performed with flounder (*Lepidopsetta bilineata*) muscle yielded almost identical results.

Minced halibut flesh was subjected to a vacuum of 3 cm. of mercury and then different lots were exposed to 30 cm. partial pressure on each of ***methyl ether, ethyl ether, methyl formate*** and ***ethyl chloride*** for 10 minutes. The direct bacterial counts after 6 days were as follows: Control (not gassed) 4,300 $\times 10^6$; methyl ether, 1,100 $\times 10^6$; ethyl ether, 1,500 $\times 10^6$; methyl formate, 110,000; and ethyl chloride, 1,500 $\times 10^6$. These results show that only methyl formate caused a marked decrease in bacterial content, the other gases having only a very minor inhibitory effect. The flesh treated with methyl formate assumed a whitish "coagulated" appearance similar to that exhibited by flesh treated with acid solutions.

DISCUSSION

The fact that the glycols studied did not prevent the multiplication of bacteria in fish flesh shows that the powerful anti-bacterial action of ethylene and propylene oxide cannot be attributed to the corresponding glycols which might be formed on hydrolysis. In this connection it must be noted that, while minute traces of the vapours or "aerosols" of some glycols are particularly toxic to certain bacteria and viruses, their solutions when in direct contact with the organisms are usually quite ineffective (Robertson, Brigg, Puck and Miller 1942; Robertson, Puck, Lemon and Loosli 1943). It is of interest that, while methyl bromide readily destroyed fish spoilage bacteria in ordinary nutrient medium, it had practically no effect on those naturally present in fish muscle. The reason for this is not clear, but it is suggested that, since methyl bromide is almost insoluble in water and highly soluble in fats, it was rapidly taken up by the latter and did not reach the organisms in lethal concentration.

It would seem that a rather high concentration of ethylene oxide or propylene oxide is required to inactivate bacteria. Thus from 0.1 to 1.0 per cent was found

necessary to cause marked reduction of viable bacteria in fish muscle, the lower concentration being sufficient in the case of fatty fish (salmon) but not adequate for lingcod. Probably the activity of these compounds when applied in gaseous form is due to their very high solubility in water, which may mean that, under the conditions of the experiments recorded in this paper, over 1 per cent of the oxide was in some cases in solution in the fish muscle fluids and in intimate contact with the bacteria. Both methyl and ethyl formate appeared to be as active in destroying bacteria in fish flesh as ethylene oxide, but caused an unnatural "coagulated" appearance in treated flesh.

With the possible exception of sodium nitrite which is probably innocuous if properly used (Tarr and Carter 1942), none of the compounds studied would seem of much value as preservatives for fresh fish. Though ethylene oxide proved a very efficient bactericide its use is not indicated because of the possibility that ethylene glycol might be formed. Propylene oxide was quite effective but it was found very difficult to remove from fish flesh, and flesh treated with it assumed a curious flavour. In this connection it must be noted that Sudendorf and Kröger (1931) found that ethylene oxide was rather slowly removed from various treated foods by ordinary aeration. It is probable that it would be much more readily removed by aeration under partial vacuum. Methyl and ethyl formates destroyed the bacteria in fish flesh very efficiently but adversely affected its appearance. Penicillic acid and 4-methoxy-2 : 5 toluquinone in 0.1 per cent concentration were not so effective as 0.02 per cent sodium nitrite and accelerated rancidity in the muscle of fatty fish.

SUMMARY

Penicillic acid and 4-methoxy-2 : 5 toluquinone in 0.01 or 0.001 per cent concentration inhibited growth of pure cultures of certain fish spoilage bacteria on laboratory media, their action being more pronounced in slightly acid than in neutral or faintly alkaline media. Neither compound in 0.01 per cent concentration was effective in retarding bacterial spoilage of halibut muscle, and in 0.1 per cent concentration retarded bacterial spoilage of coho salmon muscle only feebly.

Isolated cultures of fish spoilage bacteria on laboratory media were rapidly inactivated by treatment with methyl bromide, but treatment of fish muscle with different partial pressures of this gas under partial vacuum occasioned only a very minor reduction in its viable bacterial content.

Treatment of fish muscle with ethylene oxide, propylene oxide and methyl formate as gases under partial vacuum markedly delayed the increase of bacteria therein. When applied in solution, from 0.1 to 1.0 per cent of these compounds was required in order to markedly delay bacterial growth in fish muscle. Ethyl formate appeared to be as active as methyl formate in preventing bacterial growth, but both compounds occasioned a very marked "coagulated" appearance in treated fish flesh.

Sodium nitrite (0.02 per cent) retarded bacterial increase in fish flesh, but penicillic acid, 4-methoxy-2 : 5 toluquinone, sodium chlorite, sodium benzoate

and a patent fish preservative studied were much less effective in this respect. None of a number of other compounds studied delayed bacterial growth appreciably in fish flesh.

ACKNOWLEDGMENTS

The culture of *Penicillium cyclopium* Westling was obtained from Professor H. Raistrick of the London School of Hygiene and Tropical Medicine through the courtesy of Dr. K. P. Raper of the U.S. Dept. of Agriculture. I wish to thank Miss F. M. Lew for her technical assistance in the course of this work.

REFERENCES

ASHLEY, J. N. *J. Chem. Soc.*, **1937**, 1471-1472, 1937.
BAER, J. M. U.S. Patent No. 2,229,360, 1941.
BIRKINSHAW, J. H., A. E. OXFORD AND H. RAISTRICK. *Biochem. J.*, **30**, 394-411, 1936.
DUDLEY, H. C., AND P. A. NEAL. *Food Res.*, **7**, 421-429, 1942.
GRIFFITH, C. L., AND L. A. HALL. U.S. Patent No. 2,107,697, 1938.
GROSS, P. M., AND L. F. DIXON. U.S. Patent No. 2,075,845, 1937.
MRAK, E. M. *Rep. Ann. Date Grower's Inst.*, **18**, 3-4, 1941. (Through *Chem. Abst.*, **36**, 3863, 1942).
OXFORD, A. E., H. RAISTRICK AND G. SMITH. *Chem. and Ind.*, **61**, 22-24, 1942.
ROBERTSON, O. H., E. BRIGG, T. T. PUCK AND B. F. MILLER. *J. Exp. Med.*, **75**, 593-610, 1942.
ROBERTSON, O. H., T. T. PUCK, H. F. LEMON AND C. G. LOOSLI. *Science*, **97**, 142-144, 1943.
SUDENDORF, T., AND E. KRÖGER. *Chemiker-Ztg.*, **55**, 549-550, 570-572, 1931.
TARR, H. L. A. *J. Fish. Res. Bd. Can.*, **4**, 367-377, 1940.
J. Fish. Res. Bd. Can., **5**, 265-275, 1941.
J. Fish. Res. Bd. Can., **6**, 74-89, 1942.
J. Fish. Res. Bd. Can., **6**, 119-128, 1943.
TARR, H. L. A., AND B. E. BAILEY. *J. Fish. Res. Bd. Can.*, **4**, 327-336, 1939.
TARR, H. L. A., AND N. M. CARTER. *J. Fish. Res. Bd. Can.*, **6**, 63-73, 1942.
TARR, H. L. A., AND P. A. SUNDERLAND. *Fish. Res. Bd. Can. Prog. Rep. Pac.*, **39**, 13-16, 1939.
J. Fish. Res. Bd. Can., **5**, 148-163, 1940.
YESAIR, J., AND O. B. WILLIAMS. *Food Res.*, **7**, 118-126, 1942.

The Relation of Lake Population Density to Size of Young Sockeye Salmon (*Oncorhynchus nerka*)

By R. E. Foerster
Pacific Biological Station

(*Received for publication September 8, 1943*)

ABSTRACT

For Cultus lake, B.C., data concerning relative numbers of young sockeye inhabiting the lake each year were obtained from the count of seaward migrants. From samples of the latter the sizes of the migrants were determined. For all years, 1927-1937, a statistically significant inverse correlation of −0.815 existed between extent of lake population and size of migrants. Under natural conditions the lake's sockeye productivity seems limited, probably due to limited plankton crop, and the sockeye values obtained in each year are compared to a production level of 6,000-8,000 kg. Experiments in reducing predaceous fish populations indicate appreciable increase in sockeye production.

In the course of an extended investigation (1925-1936) of the propagation of sockeye salmon (*Oncorhynchus nerka* Walbaum) at Cultus lake, British Columbia (Foerster 1929a, 1938a), data have been assembled concerning the number of seaward migrating young each year and the mean size of the migrants. These data have been used to determine the possible influence of population density, as computed from the numbers of seaward-migrant sockeye, upon growth rate during the period of residence in the lake.

Grateful acknowledgement is made of valuable suggestions and criticisms given by Dr. William E. Ricker, University of Indiana, who was actively associated with the Cultus Lake research from 1931 to 1938.

METHODS

Lake Population Density

Although spawning of sockeye salmon occurs in the autumn (October to December) the fry do not hatch out and pass to the lake until the following spring (April and May). They remain in the deeper strata of the lake, feeding upon plankton crustacea (Ricker 1937) for approximately eleven and a half months. The majority thereupon migrate seaward from the lake, but some, the smaller ones (Foerster 1929b, 1934, 1936, 1937, and table III below), remain in the lake for an additional year, moving seaward during the following spring as two-year-olds.

J. Fish. Res. Bd. Can. 6 (3) 1944.
Printed in Canada

The lake sockeye population in any one year thus consists mainly of (1) young fish developing during the year from fry to yearling size and constituting the yearling migration, (2) young fish developing from fry to yearling size but not migrating at the end of their first year, and (3) those non-migrating yearlings which remain in the lake for a second year. There are also some sockeye that do not respond to the migratory stimulus even at the end of the second year, termed "residuals" by Ricker (1938), who found that small populations of "kokanee" or land-locked sockeye also inhabit the lake. Since neither of these groups has been found to be large and estimation of the numbers resident in the lake each year has not been possible, they have been ignored in the present study.

The sockeye population density of the lake each year is computed from the number of seaward migrants counted. Although the true population is obviously much greater at the beginning of the year and (Foerster 1938b) becomes gradually reduced to the number of actual migrants, the reductions are probably fairly proportional from year to year and therefore the number of seaward migrants may be accepted as an index of the general population. Ricker (1941) has shown that consumption of young sockeye in Cultus lake by predaceous fish is in proportion to abundance of sockeye.

While the counts of yearling migrants can be utilized directly (table IV), those for two-year-olds have to be given special treatment in order to assess their influence in each of the two years of lake residence and to express it in terms of yearlings. Survival of young sockeye from fry to yearling migrant stages has been found (Foerster 1938a) to vary from 2.81 to 5.83 per cent, the average being 4.16 per cent. While for those smaller sockeye which do not migrate at the end of the first year the survival rates would probably be even lower during the first season, they would be considerably higher for the second year because of the larger size. Therefore to make a reasonably adequate allowance for these varying rates it is assumed that the count of the two-year-old migrants occurring in any year would approximate 10 per cent of the fish present at the end of the first year as non-migrating yearlings. Each year's total of two-year-old migrants therefore has been multiplied by a factor of 10 and the product added to the previous year's yearling migrant count (table IV). While the non-migrating yearlings have a slower growth rate (table III) than the migrating individuals, the differences in size are not deemed to be sufficiently great to warrant considering the former less effective than the latter in influence on the lake's economy during the year.

For their second year in the lake, the two-year-olds are initially competing with small fry of the year, approximately 0.15 grams in weight, for food. The ratio, on the basis of weight, would probably vary from 1: 0.05 to 1: 0.02, if one considered the two-year-olds as being only slightly smaller than the average yearlings at the end of the first year. The fry grow rapidly during the summer and at migration time the mean weights of yearling and two-year-old migrants would approximate those shown in table I.

In the two years when relatively large samples were treated, 1930 and 1934, the ratios of weights between two- and one-year individuals were 1: 0.4 and 1: 0.5 respectively. In other years, however, the ratios averaged approximated 1: 0.3.

Under all these circumstances a ratio of 1: 0.1 has been selected as a reason-

able representation of the influence of two-year-old sockeye in the lake during their second year in relation to one-year fish. In other words, each two-year migrant is considered the equivalent of ten yearling migrants (table IV).

TABLE I. Mean lengths and weights of one-year and two-year-old seaward migrating sockeye, for those years when samples of two-year migrants were available.

Year	One-year individuals			Two-year individuals		
	Number in sample	Length in cm.	Weight in gm.	Number in sample	Length in cm.	Weight in gm.
1929.....	4761	6.84	3.06	30	11.76	12.64
1930.....	387	8.76	6.55	778	11.83	16.44
1931.....	719	9.08	7.10	50	13.13	22.60
1934.....	609	8.65	6.53	499	10.60	12.07
1935.....	1503	9.07	7.55	76	12.73	20.33

SIZE OF SOCKEYE MIGRANTS.

From each year's seaward migration a random sample was taken to determine length and weight frequencies and the length-weight relationship. In the first year, 1927, the samples were taken by dipping out at random a more or less similar volume of fish from the traps at several periods during the migration. In subsequent years one specimen was taken for each one hundred or two hundred individuals counted, thus obtaining a more complete representation of the migrants throughout the season.

From these data mean lengths and mean weights have been calculated and equations obtained by the method of least squares for construction of length-weight curves. Yearlings only are considered and the variations occurring from year to year in size are utilized to portray the trends in the relationship of population density to size.

MEAN SIZE OF YEARLING MIGRANTS

In figures 1 and 2 are given the weight and length frequencies of the samples of yearling sockeye salmon migrating seaward from Cultus lake, for each year from 1927 to 1937, the numbers of individuals occurring in each weight and length class being computed as a percentage of the total year's sample. The mean weight and length values are indicated by the vertical lines. In table II the means, standard deviations from the means (SD) and standard errors of the means (SE) for each year are tabulated and also the values for k and n in the equation $W = kL^n$, the equation for the length-weight curve when computed by least squares.

In general, the year-old migrants occurring in the years from 1927 to 1935 attain a fairly uniform size, around 9.0 cm. in length and from 6.5 to 7.5 grams in weight. Two notable exceptions occurred, in 1929 and 1933, when unusually heavy runs of yearlings (table IV) took place. In these two years the sizes, as revealed by the samples collected during migration, were significantly smaller,

yet as representing the mean size of yearling sockeye in the lake they are too great, for they include only the larger size classes which migrated and do not represent the smaller fish which remained in the lake for a second year. Some

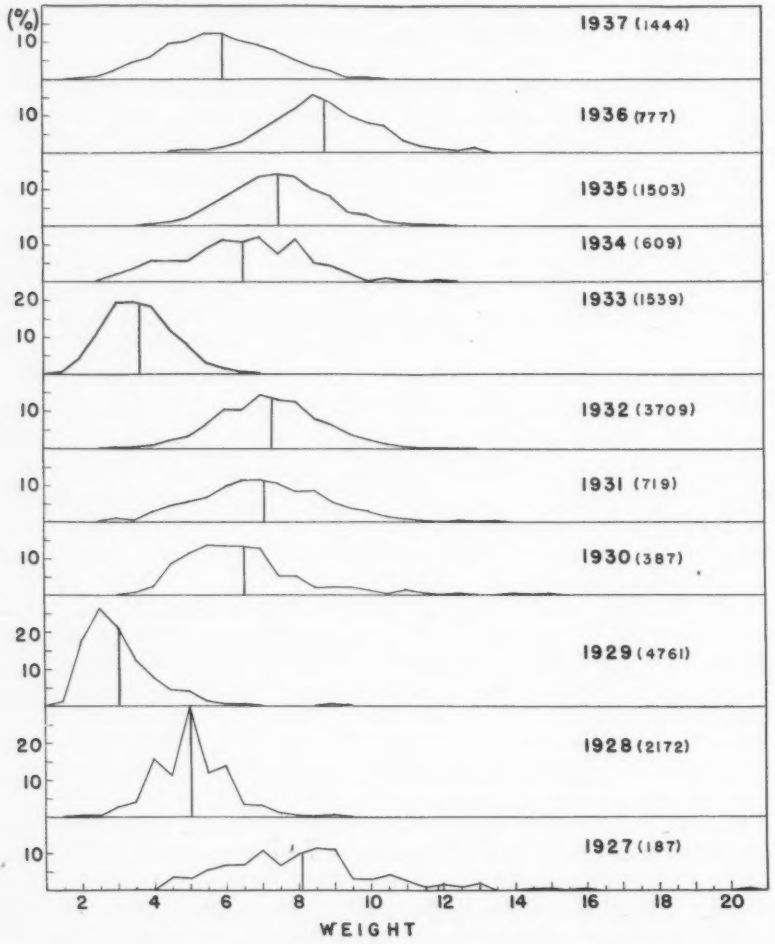


FIGURE 1. Weight frequencies of representative samples of yearling sockeye migrating from Cultus lake each year from 1927 to 1937, the number of individuals in each 0.5 g. weight group being computed as a percentage of the year's total sample. The vertical bars indicate the average weight for each year's sample.

idea of the probable difference in size is revealed by a comparison of the mean lengths at the end of the first year (first winter check), as calculated from the scales of yearlings of 1929 and 1933 and two-year-olds of 1930 and 1934 (table III).

It is assumed that the weights would have been correspondingly smaller, also, than the means shown for migrant samples.

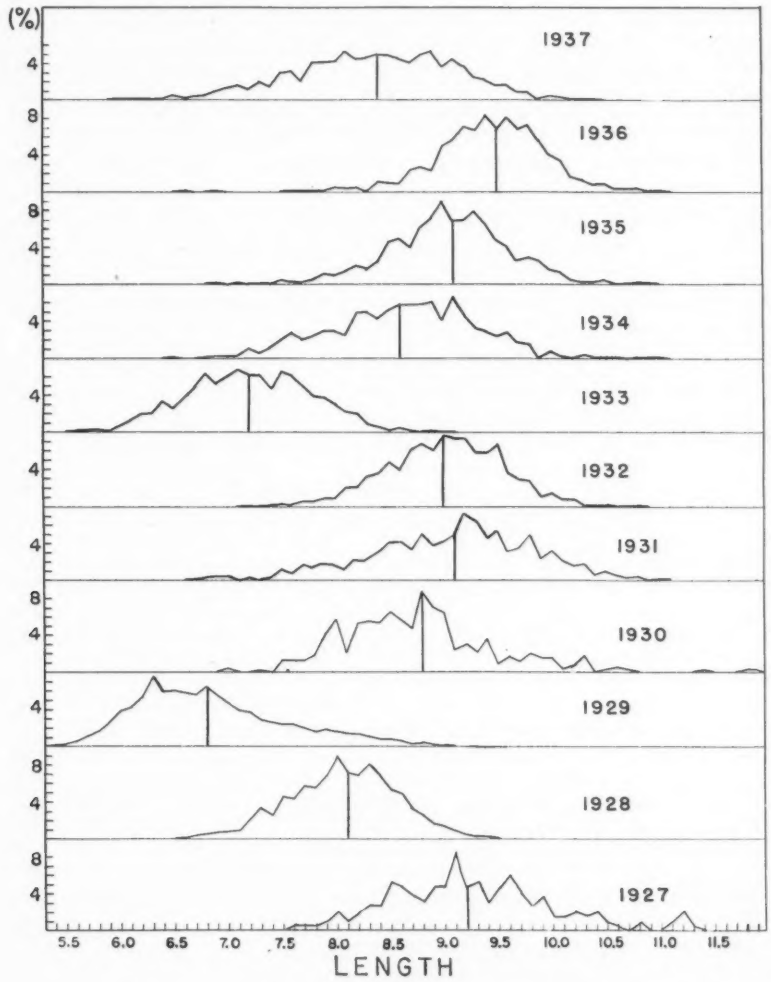


FIGURE 2. Length frequencies of representative samples of sockeye migrating from Cultus lake each year from 1927 to 1937, the number of individuals in each 0.1 cm. length group being computed as a percentage of the year's total sample. The vertical bars indicate the average length for each year's sample.

In the two following years, 1930 and 1934, the yearling migrations were quite small, 38,600 and 121,500 respectively. The presence of unusually large numbers of two-year sockeye in the lake, approximately 66,600 and 63,500 respectively, tended to compensate as far as conditions for growth in the lake

during the year are concerned, hence limited the size of the yearlings to a greater degree than the population estimates, as indicated by the migrant counts, would have suggested. The mean length and weight data for the two-year fish have been given in table I.

TABLE II. Length and weight data pertaining to each season's seaward migrating sockeye salmon. The values for k and n in the equation for the length-weight curve, $W = kL^n$, as computed by least square treatment of the data are also given.

Year of migration	Brood year	No. of indiv.	Mean length in cm.	SD.	SE.	Mean weight in g	SD.	SE.	k	n
1927...	1925	187	9.22	0.778	0.057	8.10	2.348	0.172	0.0033	3.499
1928...	1926	2,172	8.06	0.268	0.005	5.04	0.980	0.021	0.0146	2.795
1929...	1927	4,761	6.84	0.709	0.010	3.06	0.967	0.014	0.0152	2.746
1930...	1928	387	8.76	0.712	0.036	6.55	1.781	0.091	0.0270	2.521
1931...	1929	719	9.08	0.759	0.028	7.10	1.791	0.067	0.0061	3.194
1932...	1930	3,709	9.02	0.562	0.009	7.32	1.467	0.024	0.0113	2.940
1933...	1931	1,539	7.22	0.573	0.015	3.67	0.942	0.024	0.0055	3.281
1934...	1932	609	8.65	0.698	0.028	6.53	1.743	0.071	0.0041	3.407
1935...	1933	1,503	9.07	0.567	0.015	7.55	1.447	0.037	0.0093	3.039
1936...	1934	777	9.49	0.562	0.020	8.83	1.551	0.056	0.0145	2.849
1937...	1935	1,541	8.38	0.751	0.020	5.96	1.557	0.041	0.0072	3.151

TABLE III. Comparison of the lengths at the end of the first winter, as computed from the scales (Foerster 1929a) of yearling (1929 and 1933) and two-year (1930 and 1934) migrants. The numbers of scale rings occurring are also recorded.

Year	Age at migration	Number of individuals	Length at end of first year	Number of scale rings at end of first year
1929.....	I	895	62.0 cm.	8.0
1930.....	II	777	50.8 cm.	5.0
1933.....	I	302	68.0 cm.	8.4
1934.....	II	189	50.5 cm.	5.0

In the spring of 1935, it was decided to terminate the propagation study and attempt to determine to what extent reduction in numbers of predaceous fish in the lake would increase survival of young sockeye. Consequently a fairly comprehensive gill netting campaign was initiated after the seaward migration of sockeye had occurred and was continued through to the spring of 1938 (Foerster

and Ricker 1941). One effect of these operations was an increase in the growth rate of the young sockeye in the lake. This was indicated in the migration of 1936 when, with a run of 503,000 migrants (501,600 yearlings and 1,400 two-year-olds) the length and weight averages for the yearlings were the highest yet observed, 9.49 cm. and 8.83 grams respectively. It was confirmed the following year, 1937, when with a seaward migration of 3,124,000 migrants (3,101,000 yearlings and 23,000 two-year-olds), the largest migration from Cultus lake on record, the average length and weight measurements for the yearlings were 8.38 cm. and 5.96 grams, respectively. These means may be compared with those of 6.84 cm. and 3.06 grams occurring in 1929 when a relatively heavy migration of 2,464,500 migrants was counted (2,456,200 yearlings and 8,300 two-year-olds). In spite of the much larger number of migrants in 1937, the mean sizes were conspicuously greater. Because of the increased survival rates resulting from reduction of predators, a greater number of sockeye resulted from the 40,000,000 egg deposition of 1935 than from that of 250,000,000 eggs in 1927 and competition for food in the lake was less acute.

In view of these changed conditions occurring subsequent to 1935, the data for the 1936 and 1937 migrations have been separated from those of previous years in studying the size to population density relationships and the two periods treated individually.

VARIATION IN LAKE POPULATION DENSITY

In a previous section it has been indicated that the lake population density is taken to be represented by the yearling migrants counted on migration seaward and the two-year-old migrants computed to their equivalent in yearlings. In table IV are given the lake populations (column 2) from 1927 to 1938, as calculated from the actual migrant counts (columns 3 and 4), namely, yearlings + two-year-olds $\times$ 10 + two-year-olds of next year $\times$ 10.

In three of the years, 1929, 1933 and 1937, heavy migrations occurred, the result of "big" year spawnings in 1927, 1931 and 1935 respectively. In the other years the migrations varied between 400,000 and 900,000 individuals. In 1938 a relatively large migration occurred but, as explained elsewhere, this was partly due to unusual circumstances resulting from altered conditions in the lake which increased survival of sockeye quite significantly.

The purpose of the Cultus lake investigation was the study of the relative merits of natural and artificial propagation. Different methods of propagation were tested and the initial stocks of sockeye were introduced into the lake in different stages of development, naturally deposited eggs, "eyed" eggs, or free-swimming fry, as shown in column 5 of table IV. The numbers of eggs or fry handled also varied considerably from year to year. Nevertheless since in the present study only the stages from free-swimming fry to seaward migrant are of significance and it has been found (Foerster 1938a) that no significant difference prevailed in percentage efficiency between any of the propagational methods, though a certain amount of variation occurred in each of the methods, neither the original extent of planting, natural or artificial, nor the stage of development are deemed to alter appreciably the population density levels as derived from the migrants.

POPULATION DENSITY AND SIZE OF MIGRANTS

From figure 3 it is apparent that a definite inverse correlation prevails between lake population of sockeye and both mean length and mean weight of yearling sockeye migrants. For the period 1927 to 1935, i.e. prior to the commencement of predator reduction experiments and changed conditions in the lake, as discussed above, the coefficient of correlation (weight to population) is -0.815, using the factor of $10\times$ for the two-year-olds occurring in each migration.

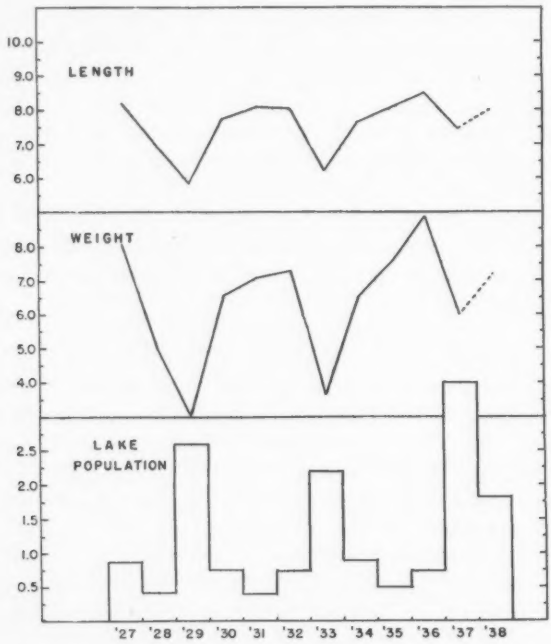


FIGURE 3. Annual trends in mean length in centimetres, and mean weight in grams of seaward migrating yearling sockeye from Cultus lake, 1927-1938, and lake population density in millions of yearling sockeye as computed in table IV. The broken lines (- - -)$^{\blacktriangledown}_{\blacktriangle}$ for 1938 indicate merely tentative averages obtained from observation only.

Attention has been directed (Hile 1936) to the relationship between densities of populations of fish and the rate of growth or size of the fish. It has been suggested that the effect on the fish may be either the result of competition for food or of the operation of a "space factor". From the present data the inference may be drawn that, for sockeye salmon, a body of water such as Cultus lake has a limited productive capacity and that size of fish is closely related to population density. When small or moderate populations are present, up to perhaps a million migrants, growth may be fairly independent of numbers present and a relatively large size, 8.5 to 9.5 cm., or 6.0 to 9.0 grams, is attained. When, how-

ever, the population is greatly increased, as the result of a heavy spawning and hatch, conditions for normal growth become taxed and a definite reduction in growth rate and size occurs.

TABLE IV. Lake population estimates as calculated from the actual yearling and two-year-old sockeye migrant counts for each year from 1927 to 1938, with information as to the method of propagation used in the brood year and the extent of the egg or fry planting.

Year	Calculated lake population	Actual migrants counted		Migrants resulting from	Brood year
		Yearling	Two-year-old		
(1)	(2)	(3)	(4)	(5)	(6)
1927.....	865,000	183,400	66,500	17,470,000 eggs deposited	1925
1928.....	436,000	336,200	1,700	5,916,000 fry liberated	1926
1929.....	2,605,000	2,426,200	8,300	250,000,000 eggs deposited	1927
1930.....	757,000	38,600	66,600	2,650,000 eggs planted	1928
1931.....	404,000	349,900	5,200	9,093,000 fry liberated	1929
1932.....	790,000	788,400	200	24,900,000 eggs deposited	1930
1933.....	2,204,000	1,571,000	0	54,300,000 eggs deposited 8,083,000 fry liberated	1931
1934.....	896,500	121,200	63,300	4,825,000 fry liberated	1932
1935.....	498,500	242,500	14,200	4,371,000 eggs planted	1933
1936.....	746,000	501,600	1,400	5,590,000 eggs planted	1934
1937.....	3,531,000	3,101,000	23,000	40,000,000 eggs deposited	1935
1938.....	1,827,000	1,627,000	20,000	12,468,000 fry liberated	1936

It is of interest to define from the data the probable productive capacity of the lake in migrating young sockeye, and in figure 4 are plotted the weight to population records of each year from 1927 to 1938 in relation to a fixed sockeye migrant production level of 6,000 to 8,000 kg. For four of the years, 1928, 1930, 1931 and 1935, the agreement of the points to the productivity band is not particularly close. They all lie below, hence it may be inferred that for some reason either the productive potentialities of the lake were not fully utilized or the productive capacity of the lake was temporarily reduced. It may be that the competitive influence of other species, perhaps predators or lake resident sockeye or coho salmon, varying in number from year to year, may have had a bearing on the general reduction of food available to the main young sockeye population.

On the contrary, the points representing the weight to population relationships for 1937 and 1938—the latter but tentatively indicated because of the lack of definite records—fall far above the selected productive capacity level. In these years, as intimated in a subsequent section, the conditions in the lake appear to have been appreciably altered by reason of certain predator control operations undertaken and the sockeye production possibilities notably increased.

It is doubtful, therefore, whether Cultus lake under normal conditions such as existed during the period of 1927 to 1935 could have supported a lake population of sockeye large enough to provide a seaward migration of more than three, certainly four million yearling migrants. At this population level the size of the sockeye would be phenomenally reduced, as will be evident from figure 5.

EFFECT OF ALTERING NUMBERS OF PREDATORS

The campaign commenced in the summer of 1935 chiefly involved reduction of the populations of the following predaceous fishes: squawfish, *Ptychocheilus oregonensis;* char, *Salvelinus malma;* and trout, *Salmo clarkii.* An early observed effect of this work was the substantially increased size of the migrants of 1936, 1937 and 1938 in relation to the extent of the migration. A further result was the increased survival rate (Foerster and Ricker 1941) among sockeye in the lake after the predator populations had been reduced.

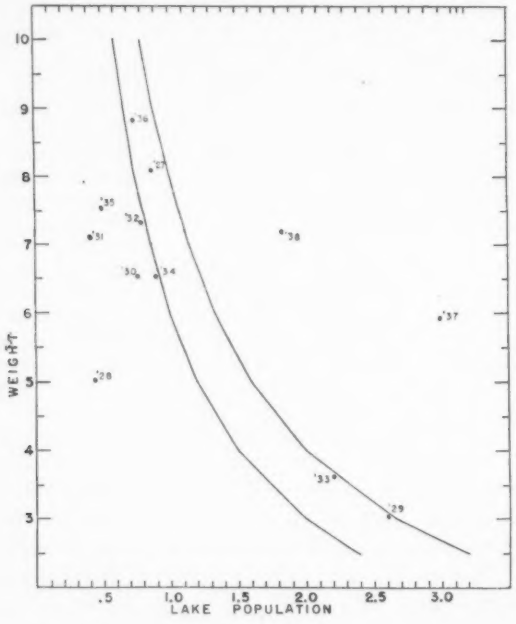


FIGURE 4. Relationships of weight, in grams, to lake population density, as expressed in millions of yearling migrant sockeye, for Cultus lake each year from 1927 to 1938, plotted in relation to a production level band of 6000 to 8000 kg. enclosed within the two curves.

The general effect of the operations was thus, as depicted in figures 3 and 4, to raise the productive threshold of the lake, as far as production of seaward migrating yearling sockeye is concerned. This is confirmed by figure 5, which shows (1) the regression line (AB) of weight of migrants on lake population, as calculated from the data for 1927 to 1935 (weight in grams $=7.2855-0.00000174$ population), (2) the line (3S) depicting the upper limit of 3 times the standard error of estimate (Chaddock 1925, p. 279) and (3) the plots of the mean weight-population records for 1936, 1937 and 1938. Since the latter points all fall well above the line 3S it is highly probable that the differences are not due to chance but represent a new relationship from that of earlier years. It is suggested that increased abundance of food per fish was probably the responsible factor.

It would have been of interest to have continued the study through succeeding years when two large spawnings occurred in successive years, 1937 and 1938, but in 1938 the Cultus lake research was taken over by the International Pacific Salmon Fisheries Commission.

CYCLIC TREND IN GROWTH

At Cultus lake there has normally occurred every fourth year, 1927, 1931, 1935, a large spawning run of sockeye which has resulted in correspondingly large seaward migrations, 1929, 1933, 1937. The migrants constituting these latter

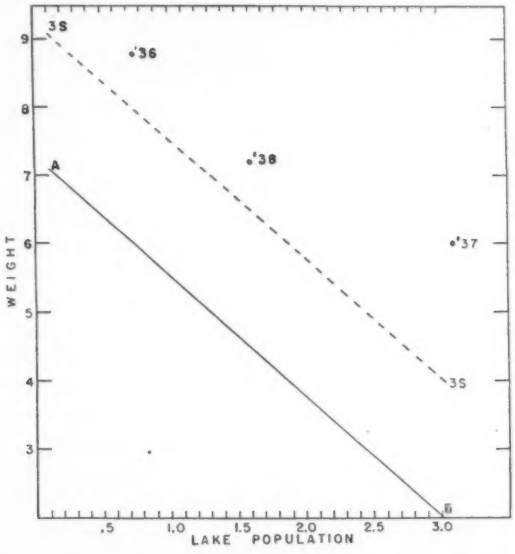


FIGURE 5. Relation of the weight-population density data for 1936, 1937 and 1938 to the regression line (AB) of weight of migrants in grams on lake population as calculated from the data for 1927 to 1935, according to the formula Weight in grams $= 7.2855 - 0.00000174$. (population). The broken line (3S) represents the upper limit of three times the standard estimate of the regression line AB.

migrations are of smaller size than in other years, presumably because of relatively crowded conditions in the lake and limited food supplies. It seemed of interest to examine the present data to determine whether this presumed drain on the lake's resources every fourth year had any effect on available food resources in succeeding years, perhaps to develop a cyclic rhythm.

It might be presupposed that any cyclic trend would be brought about by a heavy drain on the food resources of the lake during the year of heavy population and a gradual recovery of the plankton supplies in subsequent years, thus building up to a peak, as it were, in time to meet the great demand of the succeeding "big" year. This might be inferred from an analysis of figure 6 where, following the

large lake population of 1929 (2,605,000 fish; mean weight of one-year migrants, 3.06 g.), a moderate one occurred in 1930 (757,000; mean weight—6.55 g.), thereafter, in 1931, a lake population of 404,000 fish with an increase in mean weight to 7.10 g. and finally, in 1932, with a population practically doubled, 790,000 fish, the mean weight increased further to 7.32 g. Essentially the same picture is presented by the data for the 1933-1936 cycle.

However, the fact that, in figure 4, the points for the years 1931 and 1935 fall further below the selected production capacity level than those for 1930 and 1934, the years immediately following the "big" year drains, tends to weaken such cyclic interpretation. The latter may exist but be masked by other influencing

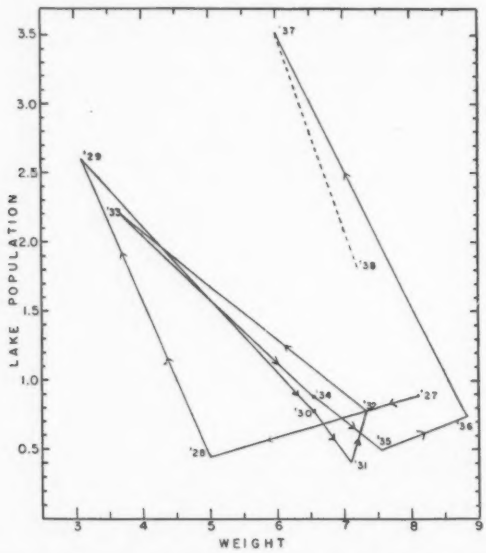


FIGURE 6. Mean weight of yearling migrant sockeye plotted against calculated lake population density to indicate the cyclic nature of the populations and the possible suggested influence of the "big" year drain on the lake's food supply on growth rate in succeeding years. The "big" years, 1929, 1933 and 1937, are considered as the first of the four-year cycle.

factors or it may be that the degree of "weighting" given to the two-year-old migrants is too high. Consideration of the effect produced by increasing or decreasing the factors used (10×) to express the two-year-old individuals in terms of yearlings indicates that any change markedly affects only the population estimates for the years following the "big" migration, namely, 1930 and 1934 when two-year-olds formed a very appreciable portion of the year's migration.

It is apparent that the data are not sufficient to clearly establish any clear-cut cyclic trend in the lake's plankton production. Therefore the problem must await further clarification.

SUMMARY

From data acquired at Cultus lake, British Columbia, from 1927 to 1938, on the number of sockeye migrating to sea each year and the mean size of the migrants it was found that:

(1) Mean length varies from 6.84 ± 0.010 cm. in the year of largest migration prior to 1937 to 9.49 ± 0.020 cm. in the year of a comparatively small migration.

(2) Mean weight similarly varied from 3.06 ± 0.014 grams to 8.83 ± 0.056 grams.

(3) While the values for the constants in the length-weight equation $W = kL^n$, obtained by the least square treatment of each year's data vary appreciably from year to year, the average for eleven years is $k = 0.0078$ and $n = 3.115$.

(4) A statistically significant inverse correlation (-0.815) exists between extent of lake population (as calculated from the seaward migrations of yearling and two-year-olds) and size of migrants, if the years of heavy migration are included. Where the populations are relatively small, between 200,000 and 800,000, a weak, positive correlation was obtained. To a certain extent, therefore, size of migrants is affected by the density of the lake population of sockeye.

(5) The weight-population relationships are plotted in relation to a yearling migrant production capacity level of 6,000 to 8,000 kg. The positions of the points suggested that this may be the general productive level of the lake. In those years where the plots fall below the production curve other factors influencing either the supply of available food or the sockeye's ability to utilize the food resource may have been responsible.

(6) Reduction in the numbers of predator fish in Cultus lake, resulting from an intensive netting campaign commenced in 1935, appreciably improved conditions in the lake and led to an appreciably higher productive capacity.

(7) The effect of the drain on the lake's productivity resources produced in the "big" year may have initiated a cyclic rhythm and influenced succeeding years' populations within the four-year cycle. The increase in mean size of migrants, correlated with increasing lake populations, may be interpreted as indicating a restoration of the productivity factors, perhaps plankton, but further data are required.

REFERENCES

CHADDOCK, R. E. Principles and methods of statistics. Houghton Mifflin Company, Boston, 471 pp. 1925.

FOERSTER, R. E. An investigation of the life history and propagation of the sockeye salmon (*Oncorhynchus nerka*) at Cultus lake, British Columbia. No. 1. Introduction and the run of 1925. *Contr. Canad. Biol. Fish.*, N.S., **4** (1), 1-35, 1929a.

Ibid. No. 3. The downstream migration of the young in 1926 and 1927. *Contr. Canad. Biol. Fish.*, N.S. **5** (3), 55-82, 1929b.

Ibid. No. 4. The life history cycle of the 1925 year class with natural propagation. *Contr. Canad. Biol. Fish.*, N.S. **8** (27), 345-355, 1934.

Ibid. No. 5. The life history cycle of the 1926 year class with artificial propagation involving the liberation of free-swimming fry. *J. Biol. Bd. Can.*, **2** (3), 311-333, 1936.

The relation of temperature to the seaward migration of young sockeye salmon (*Oncorhynchus nerka*). *J. Biol. Bd. Can.*, **3** (5) 421-438, 1937.

An investigation of the relative efficiencies of natural and artificial propagation of sockeye salmon (*Oncorhynchus nerka*) at Cultus lake, British Columbia. *J. Fish. Res. Bd. Can.*, **4** (3), 151-161, 1938a.

Mortality trend among young sockeye salmon (*Oncorhynchus nerka*) during various stages of lake residence. *J. Fish. Res. Bd. Can.*, **4** (3), 184-191, 1938b.

FOERSTER, R. E. AND W. E. RICKER. The effect of reduction of predaceous fish on survival of young sockeye salmon at Cultus lake. *J. Fish. Res. Bd. Can.*, **5** (4), 315-336, 1941.

HILE, RALPH. Age and growth of the cisco, *Leucichthys artedi* (Le Sueur), in the lakes of the northeastern highlands, Wisconsin. *Bull. U.S. Bur. Fish.*, **19**, 211-317, 1936.

RICKER, W. E. The food and the food supply of sockeye salmon (*Oncorhynchus nerka* Walbaum) in Cultus lake, British Columbia. *J. Biol. Bd. Can.*, **3** (5), 450-468, 1937.

"Residual" and kokanee salmon in Cultus lake. *J. Fish. Res. Bd. Can.*, **4** (3), 192-218, 1938.

The consumption of young sockeye salmon by predaceous fish. *J. Fish. Res. Bd. Can.*, **5** (3), 293-313, 1941.

Abdominal Width and Sexual Maturity of Female Lobsters on Canadian Atlantic Coast

By Wilfred Templeman
Memorial University College
St. John's, Newfoundland

(*Received for publication February 12, 1944*)

ABSTRACT

The relative width of the second abdominal segment was used for comparing onset of sexual maturity of female lobsters in 18 different localities of the Maritime Provinces, after establishing a correlation between them. Judging by the abdominal width, the smallest lobsters to carry eggs range from 17.6 to 22.6 cm. long, and the sizes when approximately half the female lobsters are mature range from 23.5 to 29.2 cm. in the whole southern part of the gulf of St. Lawrence and along the outer Nova Scotian coast at least as far as Jeddore; in southern Nova Scotia and Grand Manan the corresponding ranges in size are higher, being 29 to 32 cm. and 36.7 to 40.4 cm. respectively. Graphs are given showing the relative abdominal width in 189 berried lobsters.

In the formulation of laws for the conservation of the lobster, particularly for size limit regulations, a knowledge of the size at which sexual maturity occurs, which varies inversely with the temperature (Templeman 1936), is extremely important.

Since, owing to the berried lobster law and the washing of berried lobsters by some fishermen, it is extremely difficult to obtain large and random samples of berried lobsters from measurements of fishermen's catches, an easier method of estimating approximately both the smallest size at which maturity occurs and the size at which a large proportion of the population becomes mature is very useful for conservation purposes. The simplest method is measurement of the width of the abdomen of the female.

It has been demonstrated (Templeman 1935) that the relative width of the abdomen of a female lobster to the total length begins to increase with the approach of sexual maturity, this increase being rapid at first but continuing, though at a slower rate, even at the largest sizes measured.

We have therefore measured the width of the second segment of the abdomen of unselected non-berried female lobsters in eighteen different areas on the Atlantic coast of Canada (results in figs. 2, 3 and 4), the width of this segment of the abdomen being measured across the ventral surface between the spurs which form the deepest points of the pleura.

J. Fish. Res. Bd. Can. **6** (3) 1944.
Printed in Canada.

SMALLEST SEXUALLY MATURE FEMALES

From Actual Measurements

Figure 1 shows the smallest sizes of berried lobsters so far measured on the Canadian coast, consisting of results of measurements made from 1933 to 1937 as well as those previously reported (Templeman 1936, fig. 4, tab. 2).

FIGURE 1. Map of the Maritime Provinces of Canada showing in centimetres the length of the smallest berried lobsters recorded (free figures), the length at which the abdominal width of the female lobsters averages 20.6 per cent of the total length (circles), and 23.5 per cent of the total length (squares). The figure included in the square at Grand Manan is probably several centimetres too low.

Since, as noticed in 1932, large numbers of gulf of St. Lawrence lobsters maturing at a small size were being brought to Grand Manan and kept in the lobster pounds there, from which many that became berried were liberated, it has been necessary to be very careful in dealing with records of small berried lobsters from such areas of general late sexual maturity as Grand Manan.

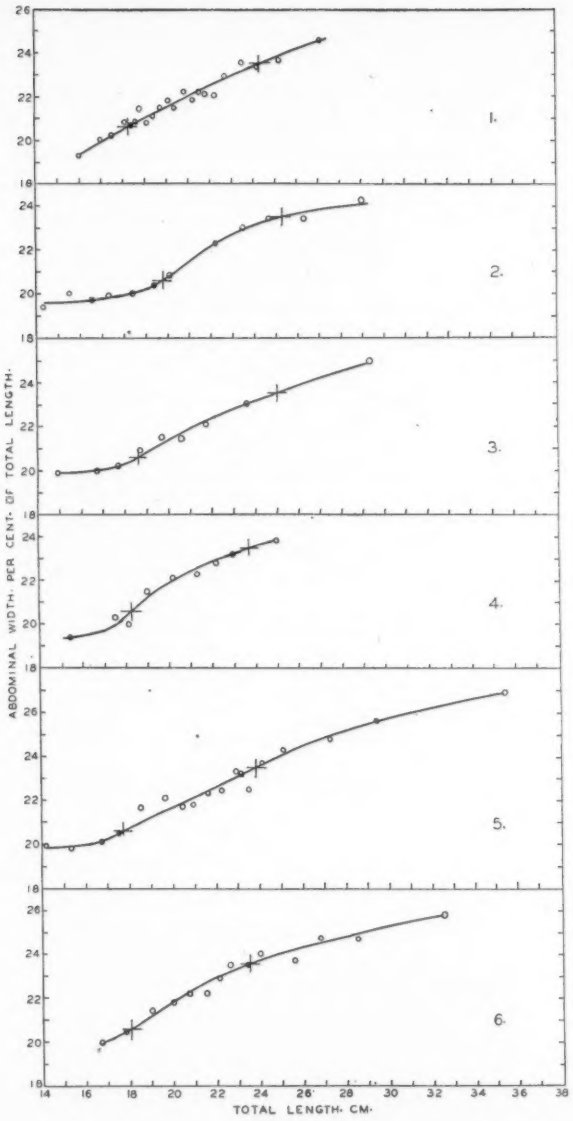


FIGURE 2. Increase in relative width of the second segment of the abdomen with increase in the total length of the female lobster; 1,—Nash creek to Green pt., June 8, 1936; 2,—Four Roads, June 11, 1936; 3,—Burnt Church, June 12, 1936; 4,—pt. Sapin, June 13, 1936; 5,—Pte. du Chêne, August to September, 1932, 1936 and 1937; 6,—cape Tormentine, September 21 and 22, 1932. Each point represents the average for 10 lobsters. At first cross width is 20.6% and at second cross 23.5% of total length.

FROM CALCULATIONS OF ABDOMINAL WIDTHS

During lobster investigation at Pte. du Chêne, N.B., 1930 to 1932 and 1936 to 1937, 228 unselected berried lobsters received directly from the fishing ground were measured, the smallest two of these being 18.0 and 17.7 cm. long respective-

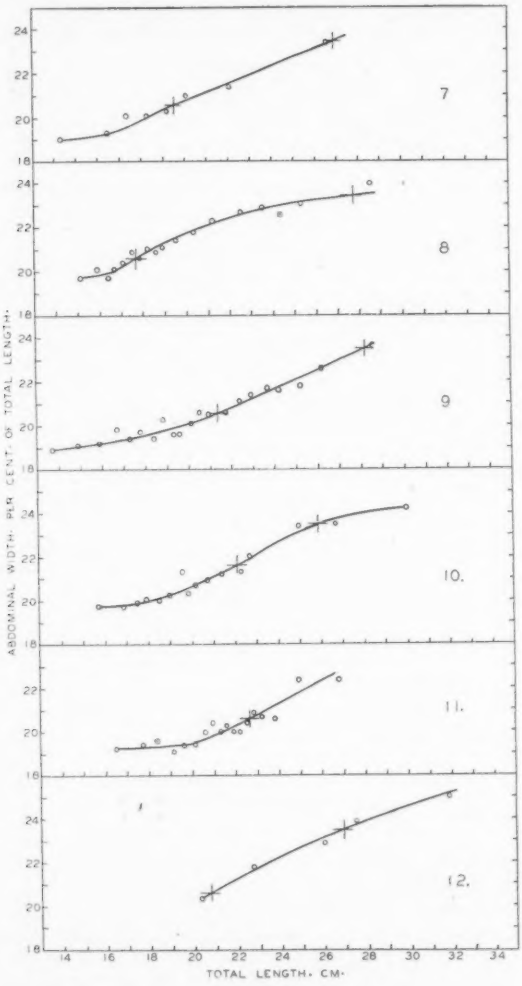


FIGURE 3. Increase in relative width of the second segment of the abdomen with increase in the total length of the female lobster; 7,—at Alberton, June 18, 1936; 8,—Caribou, June 22, 1936; 9,—Margaree island and Broad Cove Marsh, June 25, 1936; 10,—Pleasant bay and bay St. Lawrence, June 29, 1936; 11,—cape Dauphin and pt. Aconi, July 2, 1936; 12,—Canso, June 9, 1932. Each point represents the average for 10 lobsters. At first cross width is 20.6% and at second cross 23.5% of total length.

ly. According to figure 2 the average width of the abdomen of the female lobster of 17.7 cm. at Pte. du Chêne is 20.6 per cent of the total length.

We have accordingly calculated from figures 2, 3 and 4 and set out in figure 1 the sizes at which the abdominal width of the female lobsters for eighteen different

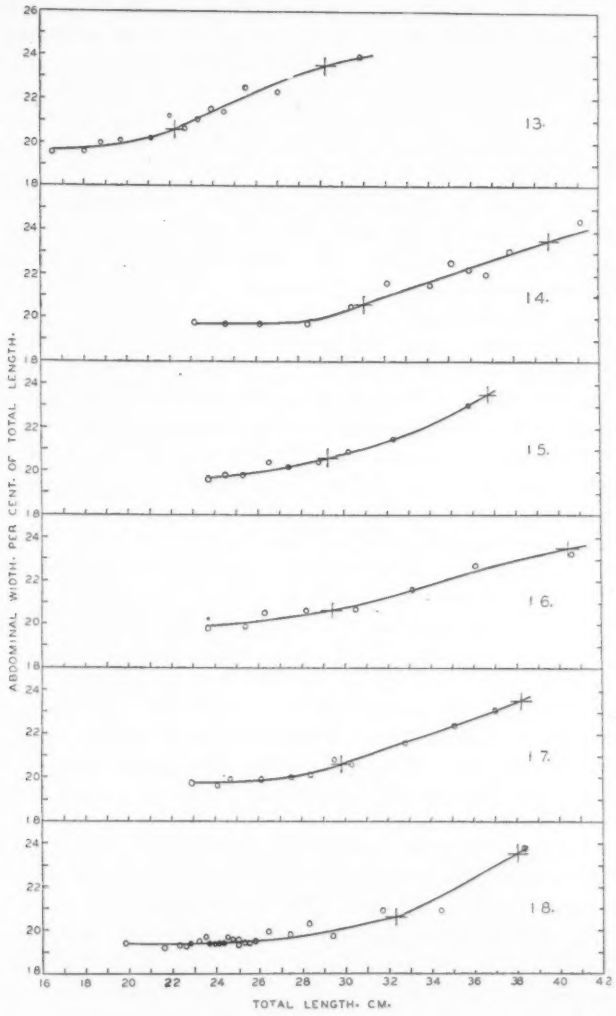


FIGURE 4. Increase in relative width of the second segment of the abdomen with increase in the total length of the female lobster; 13,—Lower East Jeddore, June 20, 1936; 14,—Seal island, May 27, 1935; 15,—Mud islands to Pubnico, June 1, 1935;. 16,—Surette's island and Tusket islands, June 3, 1935; 17,—Port Maitland, June 4, 1935; 18,—Seal cove, Grand Manan, June, 1932. Each point represents the average for 10 lobsters. At first cross width is 20.6% and at second cross 23.5% of total length.

areas around the Canadian Atlantic coast attains to 20.6 per cent of the total length. These have been accepted as the approximate comparative sizes of the smallest berried lobsters in these areas. From these calculations as shown in figure 1 the comparable smallest sizes at which lobsters begin to carry eggs are low (from 17.6 to 22.6 cm.) in the warm water of the southern part of the gulf of St. Lawrence and along the outer coast of Nova Scotia at least as far as Jeddore. In the gulf itself the size at which lobsters begin to lay eggs is smallest in the warmest areas—in the Northumberland strait, in bay Chaleur and in the Miramichi estuary; while in more exposed areas, as at Four Roads, the northern side of Prince Edward Island, and on the west coast of Cape Breton, the indicated smallest sizes of berried lobsters are from 1 to 4 cm. larger.

In the cold waters of the southern part of Nova Scotia and at the mouth of the bay of Fundy (see Templeman 1936) the smallest sizes of berried lobsters indicated by the above calculations are much larger (from 29 to 32 cm.), the largest indicated sizes being at Grand Manan.

The above calculated sizes of earliest maturity are usually slightly smaller (figure 1) than the actual measurements of the smallest berried lobsters in the same areas. Since in most areas only relatively few berried lobsters were measured, the very smallest in these areas may not have been measured.

In several localities the smallest berried lobsters as calculated are slightly larger than the smallest observed, e.g. 20.7 and 20.4 cm. respectively at Canso.

The Canso curve has been derived from relatively few measurements and possibly varies from the true curve. It might, however, quite well happen in any area that an occasional lobster remaining in some warm harbour would mature considerably in advance of the general population and before the abdomen of the average female lobster had begun to widen. We consider, therefore, that these curves of abdominal widths, when based on large numbers of measurements, and when related to the curve of abdominal width for such an area as Pte. du Chêne where large numbers of berried lobsters were also observed, offer a better comparison of the relative sizes at the beginning of sexual maturity in the general lobster population in the various areas than the small chance measurements of berried lobsters available to date. In Dawson's (Knight 1919) measurements at cape Traverse in 1918, of 374 berried lobsters of all sizes, the two smallest were between 17.1 and 18.4 cm. (6¾ and 7¼ in.) long, and were probably closer to 18.4 than to 17.1 cm. Dawson's smallest berried lobster, therefore, agrees well in length with the calculation for the smallest (18.0 cm.) in the nearby cape Tormentine area (fig. 1). Unfortunately measurements of large numbers of unselected berried lobsters are unavailable from any other Canadian locality.

SIZE FOR HALF THE FEMALES TO BE MATURE

Dawson's (Knight 1919, table III, p. 15) measurements for 3,051 female lobsters caught at cape Traverse, P.E.I., between June 24 and September 12, 1918, placed in successive half-inch size groups from 6 in. to 15 in. (15.2 to 38.1 cm.), show an increasing percentage of egg-carrying or berried individuals from the smallest upward until a length of about 12 in. (30.5 cm.) is reached,

when the numbers became somewhat small. Of the 99 above 12 in. (30.5 cm.), 25.3 per cent were berried. Considering all these to be mature, they show the full percentage of berried individuals to be expected. It may be assumed, therefore, that half this percentage, i.e. 12.6 per cent, means that half the females are mature. Knight's table shows 10.8 per cent of 444 "9 in." (22.9 cm.) females to be berried and 15.6 per cent of 411 "9½ in." (24.1 cm.). It may be concluded that at 9¼ in. (23.5 cm.) about half the female lobsters at cape Traverse were mature and able to carry eggs. The cape Traverse area where Dawson fished is

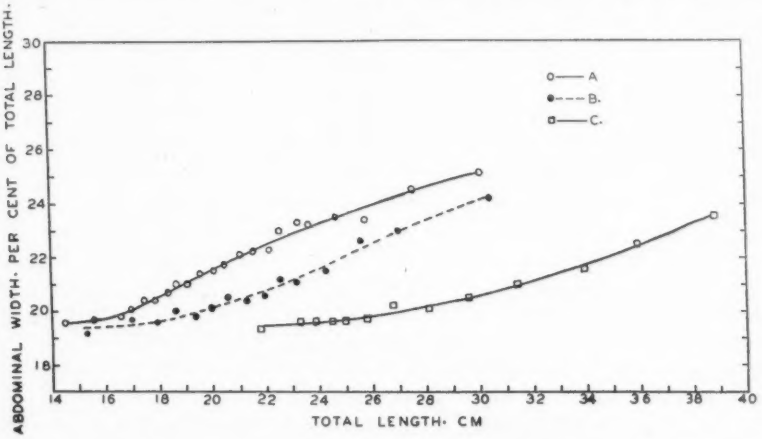


FIGURE 5. Increase in relative width of the second segment of the abdomen with increase in the total length of the female lobster; A,—from Nash creek to Caribou; B,—from Margaree island to Lower East Jeddore; C,—from Seal island off cape Sable to Seal cove, Grand Manan. Each point represents the average for 50 lobsters.

only four or five miles (6.4 to 8 km.) from the usual cape Tormentine fishing grounds in the middle of Northumberland strait off cape Tormentine. The population of lobsters in these two areas therefore is approximately the same since we have never found any significant differences in maturity and shape between lobster populations only a few miles separated and with no natural barriers between them; in fact there is little difference in these characteristics over the whole southern gulf of St. Lawrence area.

At 23.5 cm. at cape Tormentine (figure 2), the width of the second segment of the abdomen of the female lobsters is 23.5 per cent of the total length. We have noted, therefore, in figure 1 the sizes at which the width of the abdomen of female lobsters in different areas reaches 23.5 per cent of the total length and have tentatively taken these sizes as representing the lengths when approximately half the female lobsters become mature in these areas.

It will be noted that these sizes, when approximately half of the female lobsters presumably become mature, are lowest (23.5 to 29.2 cm.) in the gulf of St. Lawrence and along the Nova Scotian coast as far as Jeddore, the lowest figures for the gulf of St. Lawrence being from the warmest water in such areas

as the bay of Chaleur (24.3 cm.), and in the western part of the Northumberland strait (23.5 cm. at cape Tormentine, 23.8 at Pte. du Chêne, and 23.7 at pt. Sapin). In the more exposed areas of the gulf where the lobsters have freer access to neighbouring deeper colder water the sizes at which the width of the abdomen reaches 23.5 per cent of the total length are higher.

In the colder water areas of southern Nova Scotia and at Grand Manan the comparable sizes at which the abdominal width attains 23.5 per cent of the total length and approximately half the female lobsters are mature, are considerably larger—about 36.7 to 40.4 cm.

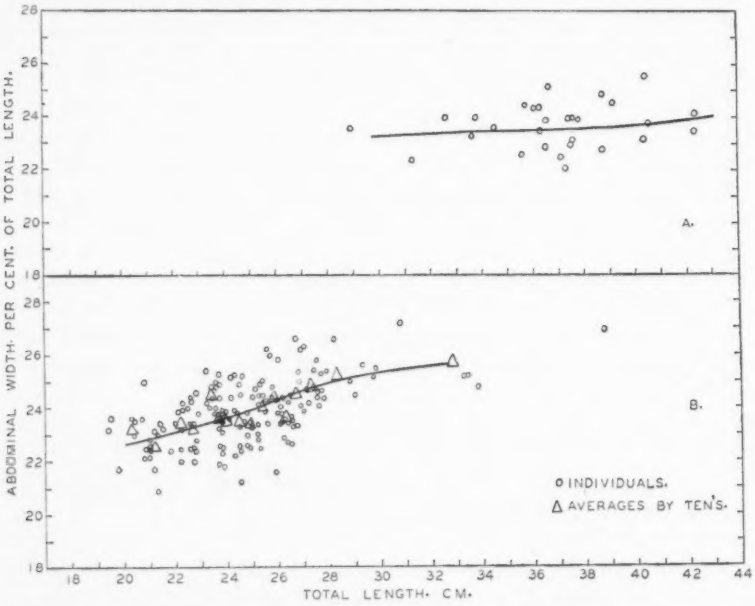


FIGURE 6. Increase in relative width of the second segment of the abdomen of berried lobsters with increase in the total length; A,—from cape Sable west to Grand Manan; B,—from bay Chaleur to Cheticamp.

Unfortunately there are no measurements of large numbers of females (including berried females) for any other locality than cape Traverse, so that the above calculations cannot at present be checked.

MAIN DIVISIONS OF COAST

On examining figure 1 it is seen that the coast investigated can be roughly divided into three regions—Nash creek to Caribou, Margaree island to Lower East Jeddore, and Seal island off Clarke's harbour to Seal cove, Grand Manan. In the first of these regions the average total length of the lobster, when the width of the abdomen equals 20.6 per cent of the total length, ranges between 17.6 and 19.8 cm., in the second between 20.7 and 22.6 cm., and in the third between

29.2 and 32.3 cm. (Undoubtedly an intermediate region between the last two exists between Halifax and Shelburne, but for this region we have no measurements of abdominal width). We have accordingly grouped together our measurements of abdominal width in each of these three areas and the results (figure 5) show that the abdomen of the female lobster widens earliest and is widest at all commercial sizes in the warm summer water of the first region, while in the colder second region it remains below that of the first region at all sizes. In the third region with high tides and lower inshore summer temperature, maturity is extremely late, with the abdomen widening significantly only at very large sizes.

ABDOMINAL WIDTH IN BERRIED FEMALES

Of 160 berried lobsters from the southern gulf of St. Lawrence (fig. 6B), only 7 possess a width for the second abdominal segment less than 22.0 per cent of the total length, only 2 less than 21.6 per cent and the lowest abdominal width is 20.9 per cent. Of 29 berried lobsters from southern Nova Scotia and Grand Manan (fig. 6A), only one possesses an abdominal width as low as 22 per cent of the total length.

In the first area with early sexual maturity (18 to 21 cm. length), the abdominal width rises fairly rapidly from an average of 22.6 per cent of the total length at 20 cm. to 25.7 per cent at 33 cm. In the second area with late sexual maturity (29 to 32 cm. length), the abdominal width rises slowly from an average of about 23.4 per cent at 33 cm. to about 23.8 per cent at 42 cm. Presumably this difference is due to the larger size at which the abdomen of these female lobsters begins to widen in the latter area, sexual maturity being so long deferred. A large increase in the actual width of the abdomen of these large lobsters might be a small increase relative to the total length of the lobster. Too few berried females, however, were measured in the latter area to make a very accurate comparison possible between the two areas. It is quite possible, however, that the abdominal width of 23.5 per cent at the size when half the females are mature, is not so accurate for the southern area as for the gulf of St. Lawrence.

SUMMARY

(1) It is concluded that the width of the abdomen of the female lobster relative to the total length can be used both for comparing the sizes at which female lobsters begin to carry eggs in various localities and also for determining the approximate size at which a certain percentage of the population of female lobsters becomes sexually mature.

(2) Since measurements of 226 berried lobsters were taken at Pte. du Chêne, the smallest being 17.7 cm. long, the average width of the abdomen of the female lobster of 17.7 cm. at Pte. du Chêne—20.6 per cent of the total length—was used for a comparison of the sizes of the smallest egg-bearing lobsters in the eighteen areas mentioned above.

(3) From these calculations the comparable smallest sizes at which lobsters begin to carry eggs are low (17.6 to 22.6 cm. long) in the warm water of the southern part of the gulf of St. Lawrence and along the outer coast of Nova

Scotia at least as far as Jeddore, and much higher (29 to 32 cm.) in the colder waters of the southern part of Nova Scotia and at the mouth of the bay of Fundy. These sizes agree fairly well with the smallest sizes of berried lobsters found in the above localities, but are naturally somewhat smaller in the areas where only a few measurements of berried lobsters have been taken.

(4) From Dawson's measurements of complete catches of 3,051 female lobsters at cape Traverse in 1918 in relation to the proportion carrying eggs, we have calculated that about half the female lobsters at cape Traverse are mature and able to carry eggs at a size of 23.5 cm. Taking the relative width of the abdomen of such lobsters as a basis, the calculated sizes when half the female lobsters are mature ranges from 23.5 to 29.2 cm. in the gulf of St. Lawrence and along the Nova Scotian coast as far as Jeddore, while in southern Nova Scotia and Grand Manan the calculated sizes are much larger, ranging from 36.7 to 40.4 cm.

(5) The coast investigated can be roughly divided into three regions—the southwest and southern part of the gulf of St. Lawrence from Nash creek to Caribou, with a high summer water temperature, and the abdomen of the female lobster widening at a small size and being widest at all commercial sizes; Margaree island in Cape Breton to Jeddore in Nova Scotia, with somewhat lower summer water temperature and the width of the abdomen less; and the southern Nova Scotia and Grand Manan area with lowest inshore summer temperatures and the abdomen of the female lobster widening significantly only at very large sizes.

(6) In the southern gulf of St. Lawrence area of early sexual maturity, the abdominal width of berried lobsters rises rapidly from about 22.6 per cent of the total length at 20 cm. to about 25.7 per cent at 33 cm., while in the southern Nova Scotia and Grand Manan area of late sexual maturity the abdominal width of the berried lobsters rises slowly from an average of about 23.4 per cent at 33 cm. to about 23.8 per cent at 42 cm.

REFERENCES

KNIGHT, A. P. Official report on lobster investigations in Canada for 1918. Can. Dept. Naval Serv., Fish. Branch, 1-35, 1919.

TEMPLEMAN, W. *J. Biol. Bd. Can.*, **1** (3), 213-226, 1935.

J. Biol. Bd. Can., **2** (1), 41-88, 1936.

The Catchability of Lobsters

By G. F. M. Smith
Banting and Best Department of Medical Research
University of Toronto

(*Received for publication April 24, 1944*)

ABSTRACT

From returns of tagging over 6,000 lobsters in the southern part of the gulf of St. Lawrence, it was found that at each of five places the percentage of tagged lobsters recaptured increased proportionally to the size, measured as log length. The differences in average size among places were significant and it was found that the regression coefficient of catchability on length varied inversely as the average size and directly as the population density. The interplay of these factors causes a decrease in average size during a fishing season and a relative increase in the catchability of the small lobsters. It is estimated that there is an economic advantage of at least six to one in favour of catching 24 centimetre rather than 17 centimetre lobsters.

INTRODUCTION

The commercial lobster fishery on the eastern coast of Canada has been exploited so intensively in the last two decades that many persons have expressed opinions as to a possible decline of the industry from over-fishing. With a view to preventing such a calamity, protective regulatory measures have been enforced by the Government for many years. The two most important, and probably most effective regulations, are the limitation of the length of the fishing season and the specification of the minimum size that may be taken. Very few people disagree with the principle of these regulations, but certainly no such unanimity can be found when the specific clauses of the regulation are discussed, such as exactly what length (or other measure of size) should be adopted for a particular place. The differences of opinion are mostly due to lack of information about the interrelationships among the many factors involved. It is the purpose of this paper to present some of the relationships among a few of the variables.

The commercial lobster fisherman is primarily concerned with obtaining the maximum amount of lobster (either by weight or by count as circumstances dictate) with the least expenditure of money and energy, and at a time when the sale of such goods will be most profitable. If we define the relative ease with which a lobster may be caught under a certain set of conditions as the catchability, the change in catchability that can be correlated with other measurable quantities is of specific interest.

In this study the catchability of lobsters was estimated by measuring, tagging with serially numbered tags and releasing samples taken with commercial fishing gear. The tagged lobsters were then subject to recapture by the com-

J. Fish. Res. Bd. Can. 6 (3) 1944
Printed in Canada

mercial fishery and a reward of twenty-five cents was offered for the return of each tag. Tagging was done before the eight-week fishing season opened and, for special purposes, during the season as well. Only returns from the year of tagging are considered, in order to reduce errors in estimates caused by loss of tags during moulting. A basic assumption made in this investigation is that tagged and untagged lobsters are caught with equal efficiency by the commercial fishery. In this study, a total of 6,058 lobsters were measured, tagged and released at six places in the southern part of the gulf of St. Lawrence. In addition to tag returns, special and detailed data on the commercial landings of lobsters by weight and size and an estimate of the fishing effort were obtained at some of the ports.

ACKNOWLEDGEMENTS

Thanks are owing to many dealers and lobster buyers in the southern part of the gulf of St. Lawrence for their assistance and cooperation in allowing the use of their records for the compilation of statistical data, and for permitting and assisting the securing and measurement of sample catches.

The work was carried out while the author was a staff member of the Fisheries Research Board of Canada, Atlantic Biological Station. All expenses were met and working facilities supplied by the Atlantic Biological Station which was under the directorship of Dr. A. H. Leim.

VARIATION OF SIZE WITH PLACE

Five lobster fishing ports in the southern part of the gulf of St. Lawrence were selected for tagging experiments. The choice of place was on the basis of average size of lobsters caught, as reported by Templeman (1936). The location of these ports, the fishing season and the mean length of lobsters in commercial catches were as follows:

Port	Location	Fishing season	Mean length (cm.) (from Templeman)
Pinette, P.E.I.	Northumberland strait	May 1-June 30	24.9
Summerside, P.E.I.	Northumberland strait	Aug. 10-Oct. 5	22.7
Shediac, N.B.	Northumberland strait	Aug. 10-Oct. 5	19.4
Miminegash, P.E.I.	West shore of P.E.I.	Aug. 10-Oct. 5	18.5
Rustico, P.E.I.	North shore of P.E.I.	May 1-June 30	17.6

In evaluating the significance of the difference between two means, it is usual to find the ratio of the difference to its standard error, which is known as t. Tables of the probablity of getting various t values by chance are available in many statistical text-books, such as Fisher (1938). The significance of differences among several means may likewise be tested by the more general method of analysis of variance. However, these methods presuppose that the variance (or its square root, the standard deviation) is independent of the mean. The mean linear measurements on lobster population samples (measured from tip of the rostrum to the end of the telson) is highly and significantly correlated with the variance. This is merely the same as saying that the greater the mean length of

a sample, the greater is the spread of these measurements about the mean. If, however, the length scale is transformed into the logarithm of length and logarithmic units used throughout, the variance (and standard deviation) is not correlated with the mean and the appropriate statistical tests of significance may be applied without bias. This procedure has been used throughout this paper.

Table I shows the mean lengths, mean log lengths and variances for the lobsters tagged and liberated and the analysis of the variance. The variance ratio F is so large that the differences among the mean log lengths of the samples could not reasonably be ascribed to sampling errors and one must conclude either that the populations from which the samples were drawn were different, or that the method of sampling has not been consistent. In the present instance the second possibility is undoubtedly true and the first may be also. The traps used at Pinette are larger and with larger openings than those used at Rustico or Miminegash. At each place the traps used for obtaining the sample were

TABLE I. Analysis of variance of lengths of lobsters tagged. L is length in centimetres and x is log length in centimetres

Place	Year	Number	Mean L	Mean x	Degrees of freedom	Sum of squares	Variance
Pinette.....	1940	1010	25.03	1.393	1009	5.192	0.00515
Summerside.....	1940	464	20.70	1.325	463	1.950	0.00421
Shediac.....	1939	1000	19.11	1.279	999	2.078	0.00208
Miminegash.....	1940	500	19.00	1.277	499	1.919	0.00385
Rustico.....	1940	959	17.57	1.240	958	4.390	0.00458
Within places....					3928	15.529	0.00393
Among places....					4	20.476	51.19
Total.....		3933			3932	36.005	

the same as traps used by the commercial fishermen to recapture the tagged lobsters, so the valid conclusion may be reached that the population of lobsters caught by commercial traps at Pinette is of greater mean log length and hence also of greater mean length than the population of lobsters caught by traps at Rustico. It will be seen that the mean lengths at each place in this study correspond rather closely with the data from Templeman (1936) shown above.

The measuring and tagging at Pinette and Rustico were done late in the fall after the fall moulting and growing season, since it would be difficult to carry out such a program before May 1 in the spring, owing to the usual ice and weather conditions. The measuring and tagging at the other three ports was done only about two weeks before the fishing season opened on August 10. The tags were small monel metal clips, bearing a serial number and were fastened through the telson as described by Templeman (1935b).

REGRESSION OF CATCHABILITY ON SIZE

The number of lobsters tagged and the number of tags returned from fishing during the next season of eight weeks, for each length class and for each

place, are shown in table II. The analysis of the dependence of catchability on size may be expressed in several forms, but the one judged to be most useful in this study was the linear relationship that was found between log length and probit capture. Log length is a mathematical transformation with which almost everyone is familiar but the transformation of a percentage scale into a scale of probits is less well known. Essentially, it is the transformation of a sigmoid curve into a straight line, or the probit is the value of the abscissa of the normal

TABLE II. The number of lobsters tagged of each length (N) and the number of recaptures (n). The figure designating length is the length class mid-point. Thus the 21 centimetre class includes all lobsters measuring between 20.5 and 21.5 cm.

Year		1940-41		1940		1939		1940		1940-41			
Length cm.	Log length	Pinette		Summer-side		Shediac		Mimine-gash		Rustico		Total	
		N	n	N	n	N	n	N	n	N	n	N	n
12	1.079									2	0	2	0
13	1.114									16	0	16	0
14	1.146					1	0	12	1	50	1	63	2
15	1.176			1	0	11	4	22	2	127	9	161	15
16	1.204	2	0	8	0	36	16	42	20	157	33	245	69
17	1.230	8	2	11	1	149	70	60	28	152	56	380	157
18	1.255	10	1	41	10	215	106	71	34	168	54	505	205
19	1.279	48	18	49	11	217	138	90	50	110	42	514	259
20	1.301	64	18	86	22	162	109	76	45	70	41	458	235
21	1.322	61	20	72	31	109	75	44	32	38	19	324	177
22	1.342	91	28	62	30	49	35	37	27	30	14	269	134
23	1.362	93	44	51	27	23	13	21	11	17	5	205	100
24	1.380	104	42	26	16	14	5	7	1	10	4	160	68
25	1.398	89	54	22	13	11	2	11	7	6	2	139	78
26	1.415	85	45	16	8	3	2	1	0	2	0	107	55
27	1.431	90	43	6	3			4	3	1	1	101	50
28	1.447	63	34	4	2			2	1	2	1	71	38
29	1.462	62	36	5	4					1	0	68	40
30	1.477	61	26	4	3							65	29
31	1.491	28	17	1	0							29	17
32	1.505	17	9									17	9
33	1.519	8	4									8	4
34	1.531	20	9									20	9
35	1.544	3	2									3	2
36	1.556	3	3									3	3
Total		1010	455	464	181	1000	575	500	262	959	282	3933	1755

or Gaussian curve where the ordinate cuts off the given percentage of the area. This transformation is convenient as each observation may be weighted in accordance with the amount of information in it by weighting coefficients that have been tabulated. Thus neither 100 per cent nor 0 per cent capture contains any information regarding the relationship between catchability and length, and 50 per cent capture contains the maximum amount of information. For details of the method of fitting the curves and the statistical theory involved, the original

papers by Bliss (1935a, 1935b) or books by Fisher (1938) and Fisher and Yates (1938) should be consulted. Bliss (1935a) and Fisher and Yates (1938) both give tables for convenient conversion of percentages into probits and the calculation of the appropriate weighting factor for fitting the best line by least squares. The two ordinate scales of figure 1 are arranged so that the percentage corresponding to any probit may be read off directly and the abscissa is scaled in both length and log length.

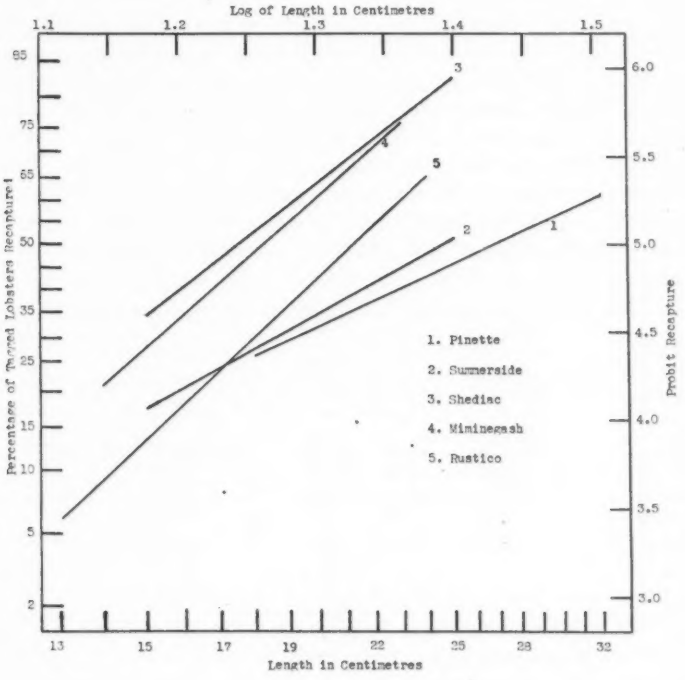


FIGURE 1

The curve fitted to the transformed observations is of the form

$$Y - \bar{y} = b\,(x - \bar{x})$$

and the confidence band about the line, of the form

$$y' = Y \pm t_{.05}\sqrt{V_{\bar{y}} + V_b\,(x - \bar{x})^2}$$

where $\bar{y}$ is the weighted mean probit capture
Y is the fitted value of probit capture
x is the log length
$\bar{x}$ is the weighted mean log length
b is the calculated slope of the line or the regression coefficient

t is the value of t at 5 per cent level for N-2 degrees of freedom, taken from a standard table of the t function (Fisher and Yates 1938)
$V_{\bar{y}}$ is the calculated variance of the mean probit capture
V_b is the calculated variance of the regression coefficient, b
P is the calculated significance level of the regression coefficient.

In calculating the regression lines, only the length classes containing 10 or more tagged lobsters were used. The weighting coefficient used in fitting the line and obtaining $\bar{x}$ depends on y as well as the class frequency. For these two reasons, the value of $\bar{x}$ is not exactly the same as the value of mean x in table I.

The calculated values of these equations are given in table III. It is seen from the P column that all the regression coefficients are highly significant. In addition to the significance of the linear regression coefficient, the goodness to fit of the data to the calculated equation was estimated by the Chi square test. All places except Rustico showed a good fit and appropriately small residual variation about the regression line (values of P from 0.93 to 0.20). The residual variation about the line for Rustico data gave a value of P from Chi square test of about 0.001. Examination of the data reveals that this is largely due to a falling off of catchability in some of the largest size classes. This may be in-

TABLE III. The regression equations of probit recapture, y on log length, x.

Place	$\bar{y}$	$\bar{x}$	b	$t_{.05}$	$V_{\bar{y}}$	V_b	P
Pinette	4.8814	1.3912	3.65	2.16	0.00169	0.41666	0.001
Summerside	4.7188	1.3237	4.37	2.31	0.00385	0.8065	0.01
Shediac	5.1987	1.2762	6.11	2.37	0.00162	1.370	0.001
Miminegash	5.0831	1.2717	7.07	2.31	0.00353	1.666	0.001
Rustico	4.44900	1.2536	7.60	2.23	0.0159	5.464	0.001

terpreted either as heterogeneity of the material or inadequacy of the fitted curve to homogeneous material. Since the type of curve fitted was adequate for the other four places and it is not shown that the material is homogeneous, the assumption of lack of homogeneity to account for the residual variation is not unreasonable. In any case the fitted regression line accounts for a highly significant part of the variability of the probit captures.

It was pointed out above that the traps at Pinette are larger than those at Rustico. In many cases the larger traps also have wider spacing of the laths. While this would undoubtedly catch a larger mean size of lobster than the smaller closer set traps, Templeman (1940) has shown that, fishing under identical conditions, the trap with wider lath setting not only catches fewer small lobsters, but catches more large. This fact should cause a great disparity between the catchability of large and small lobsters, but in this case the regression of catchability on length was smallest where it is known that the difference in method of capture was such as to exaggerate the magnitude of the regression. Thus it would appear that at least some of the differences among regression coefficients are underestimated rather than overestimated.

CHANGE IN REGRESSION OF CATCHABILITY ON SIZE

In addition to the variation in mean size from place to place, it was observed that there were some differences among places in the number of lobsters caught per trap per day during the experimental fishing. It appeared that, in general, where the lobsters were larger the average number taken per trap per day was smaller, but of the five ports, Rustico was an exception to this as a rigid rule. From known length-weight relationships reported by Templeman (1935a) the pounds per trap per day and the number of lobsters per pound could be calculated. These data are shown in table IV. The figures on pounds per trap per day are higher than is usually observed for commercial catches during a fishing season, and this is probably owing to a lack of competition for lobsters by an abundance of traps in a relatively small area. One could conclude without serious error that these figures reflect the relative population density of catchable lobsters. This would be subject to even less doubt if the traps used at each place had been identical in size, shape and construction. In the practical problem, however, these objections are not important, as it is not the population of lobsters that are catchable at all places by any particular uniform design of trap, but the lobsters that are catchable by the traps used commercially at each place that is under consideration. For our purposes we are therefore interested in the population of lobsters at each place that is subject to the commercial fishery as it is practised at present.

TABLE IV. Return for effort during experimental lobster fishing

Place	Mean log length	Observed lobsters/trap/day	Calculated pounds/trap/day	Calculated lobsters/pound
Pinette.....	1.393	2.4	2.7	0.9
Summerside....	1.325	3.0	2.3	1.3
Shediac.....	1.279	3.8	2.0	1.9
Miminegash....	1.277	3.9	2.0	1.9
Rustico.....	1.240	3.2	1.4	2.3

The variation among the regression coefficients can be shown to be significant, and it was found that these variations were correlated with both the mean log length and the average initial availability of catchable lobsters, as estimated by the number caught per trap per day during the experimental fishing. These relationships may be expressed in the form

$$B - \bar{b} = c_1 (x - \bar{x}) + c_2 (y - \bar{y})$$

where B is the estimated regression of catchability on length from x and y
$\bar{b}$ is the weighted mean regression of catchability on length for all places
x is the mean log length for any place under consideration
$\bar{x}$ is the weighted mean of the mean log lengths for all places
y is the estimate of availability from lobsters per trap per day at place under consideration
$\bar{y}$ is the mean estimate of availability for all places

c_1 is the partial regression coefficient of b on x

c_2 is the partial regression coefficient of b on y.

The calculation from the numerical values in table III and table IV gives the following coefficients and estimates of significance.

$c_1 = -15.0$ t for 2 degrees of freedom $= 4.4$ $P = 0.05$

$c_2 = 2.0$ t for 2 degrees of freedom $= 5.6$ $P = 0.05$

Total of multiple regression

F for 2 and 2 degrees of freedom $= 29$ $P = 0.05$

Thus it would appear that each of the partial regression coefficients is significant but not highly significant. The difference in catchability between large and small lobsters is greatest when the mean length is small and the population density is high.

TABLE V. Percentage of "markets" in catches and percentage of total catch by weeks.

Season.....	Spring		Spring		Fall		Fall	
Year.....	1939		1939		1939		1940	
Place.....	Pinette		Rustico		Shediac		Miminegash	
Week	Markets	Catch	Markets	Catch	Markets	Catch	Markets	Catch
1.....	85	4	11	7	8	48	10	44
2.....	79	14	10	26	10	15	10	23
3.....	77	12	9	22	11	7	12	14
4.....	82	27	7	18	10	9	12	7
5.....	54	16	7	12	8	5	13	5
6.....	63	16	5	9	7	9	14	4
7.....	48	11	0	6	7	5	11	1
8.....					7	2	7	2
Mean.....	70		7		9		11	
Total.....		100		100		100		100

CHANGE IN CATCHABILITY AND SIZE DURING A FISHING SEASON

The totals of columns in table II show that between 30 and 60 per cent of lobsters tagged were recaptured during the fishing season. Since it has been shown that large lobsters are more catchable than small, it would be expected that the mean size of lobsters caught would decrease during the season. In the southern gulf of St. Lawrence area, the catches are divided, on landing, into "market" size and "canner" size and sold by weight. "Market" size lobsters are those suitable for shipping alive to the Boston market and must be of a certain carapace length which corresponds roughly with a total length of about 23 cm. and over. "Canner" lobsters include all lobsters less than 23 cm. in

length and those few over 23 cm. that are unfit for shipment in the living state. The percentage of the landed weight that is of "market" size is therefore, a reasonably good index of the mean size. Table V shows the percentage of "market" lobsters for each week of the season and the corresponding percentage of the total catch for each week of the season for four ports.

The data for Pinette and Rustico are not for the same year as the tagging reported above, and in 1939 the fishing at each of these places was about a week late in starting, owing to ice conditions, making a seven- instead of eight-week season. At both Pinette and Rustico there is a steady decrease in the percentage of "markets" in the catches, as would be expected from the greater catchability of large lobsters. At Shediac and Miminegash (where the fishing season opens August 10 and not May 1) the picture is complicated by a lobster moulting period occurring during the season. From about the middle of August, for a period of approximately three weeks, the large lobsters are moulting, and, in September, the smaller sizes. It would appear that just previous to moulting the lobsters do not trap well because "shedder" lobsters, although taken in small numbers, are not common, and new shell lobsters are taken very readily. Thus at Shediac and Miminegash the small size of lobsters in the first weeks of the season may be due to the reduced catchability of the large lobsters that are preparing to moult. The increase in size could be caused by those lobsters entering the catch that have moulted, and incidently grown to a larger size in the process, and to the decrease in catchability in some of the smaller lobsters preparing to moult. In this area most of the moulting for all sizes is usually complete before the last week of September. The moulting periods for this area are discussed in more detail by Templeman (1934). After the differential effect by moulting on catchability is complete, the effect of size on catchability is again in evidence. The proportion of the total catch landed each week presents a different picture in the spring and fall seasons. During the fall season very large catches are the rule at the beginning, but in the spring season the catches are small at first, probably on account of the very cold water. As the water becomes warmer the catches increase for a time and then decrease. The decrease in catchability for unit effort may be ascribed to the effect of the decrease in population density by the previous fishing.

During the fishing season there is a decrease both in the size of lobsters caught and in population density. The former should increase and the latter decrease the regression coefficient of catchability on length. The change in the coefficient during a season could be calculated from the tagging already reported, if the time of capture of lobsters was known. Unfortunately these data are subject to errors in reporting of unknown magnitude, as the tags may be held for a week or more by some fishermen before being exchanged for the reward. In view of this difficulty it was thought advisable to do some special tagging during the fishing season. In this way the returns for the whole season could be compared with those for the last two weeks or the last four weeks of the season. At each of four ports 100 lobsters were measured and tagged at each of several times during the season. For present purposes the sizes are merely recorded as "market" or "canner". The proportion "canner" size recaptured, divided by the proportion of "market" size recaptured gives the catchability ratio of small

to large lobsters averaged over a period during which they were free. These data are shown in table VI. The standard errors of the catchability ratios are close to 0.12 or 0.13, so that on the average a difference between two ratios of about 0.35 would attain significance at the 5 per cent level (P = 0.05). At least for Pinette and Tormentine (in the strait of Northumberland) the relative increase in catchability of small lobsters as the season progresses is highly significant. The data for Shediac and Rustico, while not as convincing statistically, still shows a trend in the same direction. It would appear that the effect of change of population density level has been strong enough at least to counteract (Shediac and Rustico) or to override (Pinette and Tormentine) the effect of decreasing average size on the relative catchability of large and small lobsters.

TABLE VI. Recapture during the fishing season of lobsters tagged and released on the dates given, all for 1941

Recaptured up to June 30, Pinette

		May 7	May 14	May 21	May 28	June 4	June 18
Tagged	Markets	88	93	87	83	90	90
	Canners	12	7	13	15	10	9
	Markets	54	60	40	45	37	6
Recaptured	Canners	5	4	6	9	4	1
Ratio		.68	.89	1.00	1.11	.98	1.67

Recaptured up to June 30, Rustico

		May 2	May 9	May 17	May 23	June 6	June 13	June 21
Tagged	Markets	23	7	23	19	24	18	5
	Canners	119	93	77	81	76	82	83
Recaptured	Markets	13	4	12	10	8	16	2
	Canners	44	27	30	33	21	36	27
Ratio		.65	.51	.75	.78	.83	.50	.82

Recaptured up to October 5, Shediac

		Aug. 19	Aug. 26	Sept. 3	Sept. 9
Tagged	Markets	40	18	15	16
	Canners	60	82	85	94
Recaptured	Markets	18	10	8	6
	Canners	15	24	26	23
Ratio		.55	.53	.57	.73

Recaptured up to October 5, Tormentine

		Aug. 14	Aug. 21	Sept. 4	Sept. 19
Tagged	Markets	52	51	35	65
	Canners	48	49	63	35
Recaptured	Markets	14	16	9	9
	Canners	7	14	16	6
Ratio		0.54	0.91	0.99	1.24

DISCUSSION

The above data have shown that large lobsters are more catchable than small, and although the difference is not so marked when the average size of the lobsters is large, it is increased if the population density level is increased. It may be found from figure 1 or calculated from table III that a lobster 24 cm. long is about twice as readily captured as one 17 cm. long. From figure V of Templeman (1935a) it may be seen that the larger of the above weighs about three times as much as the smaller. Up to 1941, "market" size lobsters (about 23 cm. and over) sold for about twice the price per pound of the smaller size. In 1943 the difference in price was very small or in some places all sizes sold at the same rate per pound. However, this is very probably due to wartime conditions with few fishermen and a demand for lobsters. Even if the price factor is neglected it would appear that, per unit weight landed, the larger size shows an economic advantage of about six to one. This advantage would be further increased if the smaller size were not landed but allowed to remain in the sea to grow larger. The growth from 17 to 24 cm. would be completed in three moulting periods or about one year and a half. (Templeman 1940).

Since the recaptured tagged lobsters were sampled from the population of tagged lobsters by the same method that the tagged lobsters were sampled from the lobster population in the sea, it is reasonable to suppose that the relative number of lobsters of each size in the sea could be estimated. For example, at Shediac (table II) about ten times as many 17 cm. lobsters were caught as 24 cm. lobsters but the latter are twice as catchable, making the ratio in the sea about twenty to one. This type of calculation may be applied to all sizes at each place by the use of the equations in table III. Since two sampling processes would be involved in such a computation, the limits of confidence would be somewhat wider than indicated for the single sampling of the regression equations.

REFERENCES

Bliss, C. I. *Ann. Applied Biol.*, **12**, 134-167, 1935a.
Ann. Applied Biol., **12**, 307-333, 1935b.

Fisher, R. A. Statistical methods for research workers (7th ed.). Oliver and Boyd, Edinburgh, 1-356, 1938.

Fisher, R. A., and F. Yates. Statistical tables. Oliver and Boyd, Edinburgh, 1-90, 1938.

Templeman, W. *Bull. Biol. Bd. Can.*, **43**, 1-13, 1934.
J. Biol. Bd. Can., **1** (3) 213-226, 1935a.
J. Biol. Bd. Can., **1** (4) 269-278, 1935b.
J. Biol. Bd. Can., **2** (1) 41-88, 1936.
Nfld., Dept. Nat. Res. Serv. Bull. **15**, 1-42, 1940.